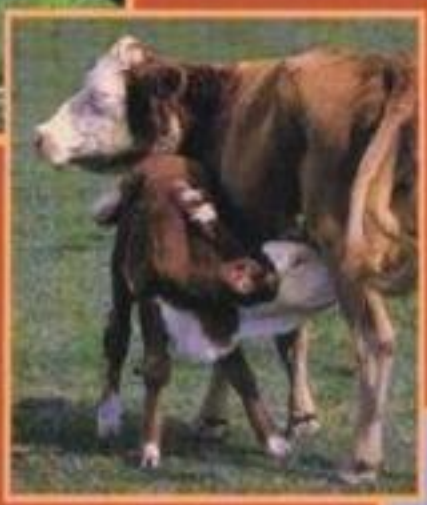
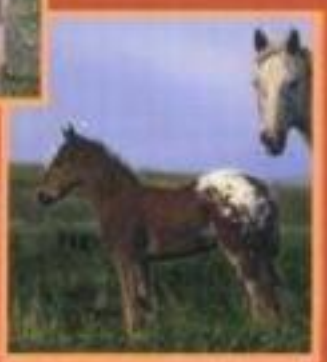
Current Therapy in

LARGE ANIMAL THERIOGENOLOGY 2





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Preface

This edition of *Current Therapy in Large Animal Theriogenology* is a continuation of the long-standing tradition of providing a theriogenology text that summarizes the reproductive information available for the large animal species. Included in this text are the equine, bovine, caprine, ovine, swine, camelid, and deer. The veterinary practitioner as well as students and academic clinicians will find this an excellent reference for their quest for knowledge.

Until recently, the importance of theriogenology to the veterinary practitioner was often overlooked, because their situation was usually not life threatening except perhaps for obstetrics or severe uterine infections. However, today its relevance to the livestock producer and large animal companion owner has become more significant. Owners demand more from their veterinarians with respect to technology and procedures such as embryo transfer, oocyte fertilization, semen freezing, shipped semen, timed inseminations, maintained reproductive performance, and so forth. These topics and more are addressed with the latest in pertinent information.

Veterinary students and academic clinicians will likewise find this textbook very informative and an invaluable aid in both their classroom and clinical work. The information presented for each species includes reproductive anatomy, physiology, methods of pregnancy diagnosis, breeding soundness examinations, surgical procedures, obstetrical procedures, and other theriogenology techniques that would be appropriate for various conditions.

Chapters are specifically devoted to each of the numerous species addressed herein. The material for this textbook was drawn from the expertise of many professionals for the benefit of its readers. We wish you only EXCELLENCE!

Walter R. Threlfall, DVM, MS, PhD
Theriogenologist

CHAPTER 1

Physiology and Endocrinology of Stallions

TRACEY CHENIER

ANATOMY AND PHYSIOLOGY

Normal spermatogenesis in stallions is a complex event, requiring a functional hypothalamic-pituitary-testicular (HPT) axis and a normal testicular environment capable of producing and supporting specific regulators of spermatogenesis (Fig. 1-1). Briefly, the anatomic features involved in the reproductive physiology of stallions include the pineal gland, hypothalamus, pituitary gland, and testis. For a detailed review of reproductive anatomy and spermatogenesis, the reader is referred elsewhere.[1,2]

The **pineal gland** is located between the cerebral hemispheres and lies dorsal to the pituitary gland. This gland produces the hormone melatonin in response to visual light signals it receives from the retina. In long-day breeders such as horses, an increase in duration of light exposure results in a decrease in melatonin production by the pineal gland. Melatonin is released in the greatest quantities during the hours of darkness. High levels of melatonin have the effect of lowering levels of gonadotropin-releasing hormone (GnRH), possibly through an influence of the feedback mechanisms exerted by androgens on GnRH release.[3] Located at the base of the brain, the **hypothalamus** produces GnRH in a pulsatile manner in response to a variety of stimuli. Olfactory, tactile, auditory, and visual signals alter the production of GnRH by the hypothalamus, which is transported via portal vessels to the anterior **pituitary gland.** Pulsatile secretion of GnRH stimulates the production and pulsatile release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the pituitary.

Within the testis, the **seminiferous tubules** of the adult stallion make up about 70% of the testicular parenchyma. The tubules consist of **Sertoli cells** and germ cells in various stages of development. The Sertoli cell is both a structurally and physiologically supportive cell to the process of spermatogenesis. As shown in Figure 1-2, developing sperm cells are embedded into, or in intimate contact with, the Sertoli cells throughout maturation. Most of the remainder of the testis consists of **Leydig cells,** myoid cells, and blood vessels of the interstitial compartment.

In adult stallions, pituitary LH binds to Leydig cell receptors, stimulating the production and release of estrogens and testosterone by the cells. Pituitary FSH stimulates the production and release of multiple factors from the Sertoli cells of the testis, including inhibin, activin, androgen-binding protein (ABP) and insulin-like growth factor (IGF). These testicular hormones and proteins feed back to the hypothalamus to regulate the pulsatile release of GnRH and therefore the continued production of LH and FSH. Estrogens, not testosterone, produced by the Leydig cells appear to regulate LH release from the pituitary gland of the stallion, either at the level of the hypothalamus by altering GnRH pulse amplitude and frequency, or directly at the level of the pituitary.[4] Treatment of long-term geldings with estradiol resulted in increased LH levels and decreased FSH levels, suggesting that estrogens may also play a role in modulating FSH secretion.[5] Because testosterone, inhibin, and FSH levels follow similar season patterns, with levels of all three hormones highest during the breeding season and lowest in the nonbreeding season, it has been suggested that inhibin may act together with testosterone to regulate FSH secretion by the pituitary.[6] Testosterone also exerts a negative feedback mechanism on hypothalamic production of GnRH. Normal function of these many complex feedback mechanisms is critical if spermatogenesis is to proceed normally.

Androgens synthesized by the Leydig cells under the influence of LH diffuse into the blood and lymph surrounding the seminiferous tubules, becoming bound by ABP. In order for normal spermatogenesis to occur, high local concentrations of androgens, especially dihydrotestosterone (DHT), must bathe the tubules. Junctional complexes between adjacent Sertoli cells form the functional blood-testis barrier, ensuring a specific physiologic environment for the developing germinal cells on either side. The blood-testis barrier also serves to isolate diploid spermatogonia and spermatocytes from the haploid postmeiotic spermatocytes, spermatids, and spermatozoa. Myoid cells lie within the lamina propria of the tubule, just beneath the basal compartment. Containing actin filaments within their cytoplasm, these cells play a role in transport of spermatozoa toward the lumen of the tubule. The paracrine factors transforming growth factor (TGF-β) and PmodS, produced by the myoid cells under testosterone influence, may regulate Leydig cells through ABP actions and modulate Sertoli cell functions.[6,7] Myoid cells may also contribute to the stability of the blood-testis barrier.[8] Recently, the degeneration and transformation of myoid cells into fibroblasts was demonstrated in the testes of a group of aged (23–24 years) stallions.[9] These findings suggest that myoid cells may play a significant role in the progression of testicular fibrosis in aged stallions and perhaps also in stallions with testicular degeneration.

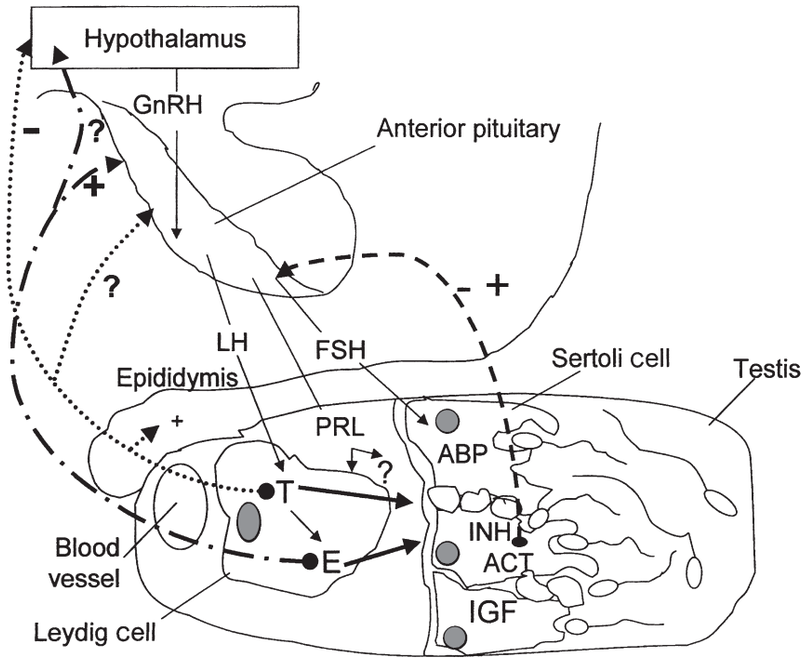

Fig. 1-1 Equine hypothalamic-pituitary-testicular axis. Hypothalamic gonadotropin-releasing hormone (GnRH) regulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary. The major regulator of Leydig cells is LH. The major products of Leydig cells are testosterone (T) and estrogens (E) in adult stallions. The major regulator of the Sertoli cells is FSH. Androgen-binding protein (ABP), inhibin (INH), activin (ACT), and insulin-like growth factor (IGF) are products of the Sertoli cells. The role of the pituitary hormone prolactin (PRL) on testicular function is unknown. Testosterone produced by the Leydig cells feeds back on the hypothalamus to inhibit GnRH production. Estrogen modulates GnRH-induced LH release. The roles of T and E in modulation of FSH release from the pituitary are unknown. Inhibin inhibits FSH release from the pituitary. Activin has been shown to positively modulate the release of FSH at the level of the pituitary in other species, but its action in the horse is unknown. (From Roser JF: Reproductive endocrinology of the stallion. In Samper J (ed): *Equine breeding management and artificial insemination.* Philadelphia: WB Saunders, 2000.)

PARACRINE AND AUTOCRINE FACTORS

A paracrine factor is a product of a cell that acts locally on another nearby cell to modulate functions. An autocrine factor is a cell product that acts directly back on the cell that produced it. Testosterone, TGF-β, IGF, inhibin, and activin are some of the paracrine factors produced by the Sertoli cell that are known to regulate spermatogenesis in other species (Fig. 1-3).[10] A growing body of evidence suggests a strong role for paracrine-autocrine factors in the initiation and maintenance of spermatogenesis in stallions as well. In a recent study of stallions aged 6 months to 23 years, plasma and testicular levels of IGF-I were significantly higher in colts younger than 2 years of age than in older stallions.[11] These results suggest an involvement of IGF-I in testicular development and the onset of spermatogenesis. No significant differences in IGF-I levels were found among stallions over 2 years of age. No association of IGF-I levels with declining fertility was found. IGF-I levels were found to be higher in the breeding season than the nonbreeding season, but the authors cautioned that the separation of age and seasonal effects in the young stallions in the study made interpretation of this finding difficult. Inhibin, a glycoprotein hormone, has been shown recently to be produced by both Sertoli and Leydig cells in stallions.[12,13] Although inhibin appears to act together with testosterone to regulate FSH secretion at the level of the pituitary gland, research in subfertile and infertile stallions suggests an important local autocrine role as well. Testicular inhibin concentrations decline in stallions with poor fertility before levels of other testicular hormones are altered,[14] suggesting that a local defect in Sertoli cell function occurs first in stallions with declining fertility.

EFFECTS OF SEASON ON REPRODUCTIVE PARAMETERS IN STALLIONS

The effects of season and day length are not as dramatic in stallions as in mares. However, stallions do experience a circannual rhythm, with testicular size, semen production, libido, and hormone concentrations varying by season. An increase in the duration of light exposure results in decreased melatonin production by the pineal gland, leading to increased GnRH production by the hypothalamus. Increasing GnRH levels stimulate

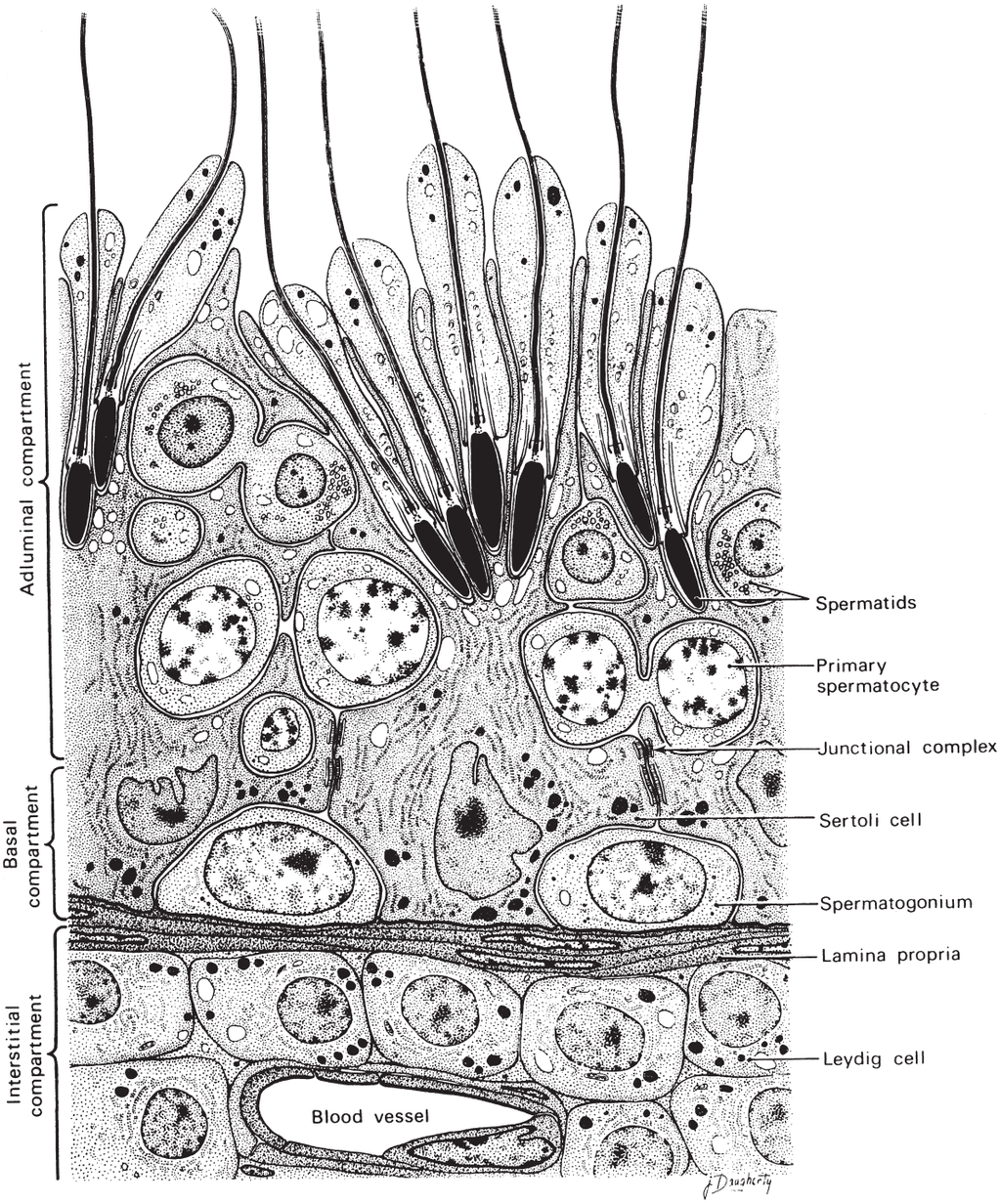

Fig. 1-2 Drawing of a section of a stallion seminiferous tubule showing the relationship of germinal cells and adjacent Sertoli cells in seminiferous epithelium. Spermatogonia, primary spermatocytes, secondary spermatocytes, and spherical spermatids all develop in the space between two or more Sertoli cells and are in contact with them. Primary spermatocytes are moved, by the Sertoli cells, from the basal compartment through the junctional complexes and into the adluminal compartment. During elongation of spermatids, they are repositioned by the Sertoli cells to become embedded within long pockets of cytoplasm of individual Sertoli cells. Note the intercellular bridges between adjacent germinal cells in the same cohort or generation. (From Pickett BW: *Management of the stallion for maximum reproductive efficiency II.* Animal Reproduction Laboratory Bulletin No. 05. Fort Colins, CO, 1989.)

increased LH and FSH production by the pituitary and corresponding increases in testosterone, estradiol, and inhibin levels. The result is an increase in testicular size and weight, sperm production, and libido during the breeding season. The release of another pituitary hormone, prolactin, is suppressed by melatonin, resulting in high plasma levels of prolactin during the long days of the breeding season in stallions. Although the role of prolactin has not been elucidated in the stallion, work in other species has shown that together with LH,

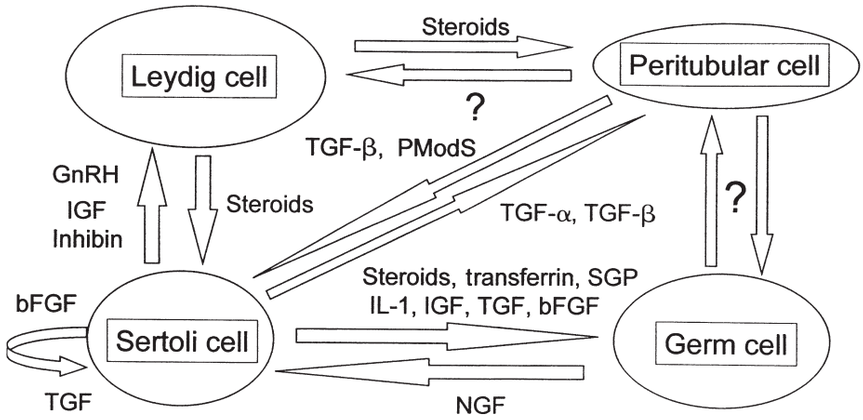

Fig. 1-3 Paracrine-autocrine regulation of testicular function. Many of the local interactions in the equine testis are unknown. The figure presents a hypothetical paracrine-autocrine system based on what has been observed in other species. GnRH, gonadotropin-releasing hormone; IGF, insulin-like growth factor; bFGF, basic fibroblast growth factor; TGF, transforming growth factor; TGF-β, transforming growth factor beta; TGF-α, transforming growth factor alpha; IL-1, interleukin 1; SGP, sulfated glycoprotein; NGF, nerve growth factor; PmodS, a nonmitogenic paracrine factor produced by peritubular cells that modulates Sertoli cell function. (From Roser JF: Reproductive endocrinology of the stallion. In Samper J (ed): *Equine breeding management and artificial insemination.* Philadelphia: WB Saunders, 2000.)

prolactin controls testicular LH receptor expression and activates androgen synthesis within the testis.[15] Hypothalamic inhibition of prolactin release by the pituitary gland appears to be determined by dopaminergic systems; dopamine inhibits prolactin release in all species studied to date. An opioidergic inhibition of GnRH/LH release may be partially responsible for seasonal effects in stallions. In contrast to rams and hamsters,[16] administration of the opioid antagonist naloxone induced LH release, followed by an increase in testosterone, in stallions during the nonbreeding season, but not during the breeding season.[17,18] Geldings treated with naloxone during or outside the breeding season did not demonstrate any differences in LH production. Treatment of long-term geldings with testosterone demonstrated that the hypothalamic and pituitary feedback mechanisms sensitive to testosterone switch from a negative to positive effect between the nonbreeding and breeding seasons.[19] It appears that the effects of photoperiod are dependent upon gonadal steroid feedback mechanisms, and the presence of gonads is required for seasonal effects on hypothalamic and pituitary hormones to be seen.

The endogenous reproductive rhythm of stallions can be only partially controlled by lighting conditions. Stallions maintained on continuous short days (8 hours of light: 16 hours of darkness) for 20 months continued a normal pattern of changes in reproductive parameters during the breeding season.[20] Exposure of stallions to continuous long days (16:8), beginning in December (Northern Hemisphere) following a period of short days, results in an earlier attainment of peak reproductive characteristics than stallions exposed to normal environmental conditions, clearly demonstrating that seasonal reproductive recrudescence is photoinducible in stallions. However, stallions exposed to extended periods of long days appear to become refractory to light. Peak reproductive characteristics are not maintained indefinitely, with testicular regression occurring despite continued exposure to long days. This photorefractory state is an important consideration in the management of stallions servicing mares in both Northern and Southern Hemispheres. Such stallions should be exposed to several weeks of short days at the end of one breeding season before being exposed to the long days of the breeding season in the other hemisphere, to ensure a "resetting" of the circannual rhythm.

HORMONAL IMBALANCES IN THE STALLION WITH IDIOPATHIC SUBFERTILITY AND INFERTILITY

The nature of reproductive dysfunction in stallions leading to idiopathic subfertility and infertility is poorly understood. There are many known causes of poor fertility in stallions, including tumors, history of steroid treatment, trauma, illness, and poor management. However, many times an examination of a stallion with poor fertility results in no evidence of the causal factor(s). Many of these stallions demonstrate hormonal imbalances, usually beginning with increasing levels of FSH, followed later by decreasing estradiol, inhibin, and LH levels, and finally, decreasing levels of testosterone and significant reductions in spermatozoal production. These stallions typically show a decrease in testicular volume, with small, firm testes found on palpation. Semen evaluation may demonstrate low total sperm numbers, increased numbers of morphologically abnormal sperm, and low motility. Findings vary depending on the stage and degree of progression of the disease process. Attempts have been made to identify these stallions early on in the disease process using hormonal testing; however, clinical experience with hormonal therapies aimed at reestablishing a normal hormonal environment for spermatogenesis have been disappointing. The observa-

tions of hormonal aberrations in subfertile and infertile stallions suggest that both a pituitary and testicular disorder may be present in affected stallions. Recent evidence suggests that the primary site of the disorder in affected stallions is the testis itself, and not the HPT axis. As mentioned earlier, intratesticular inhibin concentrations decrease before testicular and circulating levels of the pituitary hormones FSH and LH are altered, suggesting that Sertoli cell dysfunction occurs first.[14] Declining inhibin levels lead to the observed increases in FSH through reduced negative feedback influences at the pituitary gland. The presence of a circulating biologically inactive LH isoform in affected stallions has been described.[21] Blanchard and associates (1995)[22] also found differing levels of LH isoforms in fertile versus subfertile stallions. A biologically inactive form of LH may occupy Leydig cell receptors competitively and not permit adequate testosterone production within the testis to support normal spermatogenesis. Receptor-binding kinetic studies demonstrated similar receptor affinity constants and no differences in LH receptor numbers per mole of testis weight among fertile, subfertile, and infertile stallions.[23] These results suggest that defects in local postreceptor mechanisms within the testis may be involved in subfertility.

Fertile and infertile stallions appear to differ in the response of the HPT axis to hormonal challenge. Treatment of normal stallions with human chorionic gonadotropin (hCG) resulted in an increase in testosterone and estrogen levels as expected, and a decrease in inhibin.[24] Infertile stallions treated with hCG in the study demonstrated significantly lowered testicular responsiveness, with minimal changes in testosterone and estrogen levels after treatment, and no changes in inhibin levels. The results of subsequent studies show that the lowered responsiveness likely originates at the level of the testis. No significant differences were found in pituitary gland function among fertile, subfertile, and infertile stallions 1 year following castration.[25] In a second study conducted 1 year following castration of a group of fertile, subfertile, and infertile stallions, no differences among stallions were observed in pituitary response to steroid replacement therapy.[26] Steroid replacement therapy increased plasma testosterone and estradiol concentrations in all three groups, and no significant differences in LH or FSH levels among the groups of stallions were found before or after treatment. In all three groups of stallions, estradiol treatment significantly increased plasma LH levels and decreased FSH levels, but testosterone treatment decreased LH levels and increased FSH. In addition, fertile, subfertile, and infertile stallions responded similarly to GnRH challenge, with no differences in LH or FSH levels after GnRH stimulation among the groups 1 year after castration. Taken together, these recent studies indicate that removal of the testes removes the problem, allowing the hypothalamic-pituitary axis to return to normal. This suggests that the testis likely represents the primary site of disorder in stallions with idiopathic infertility. However, the exact nature of the disorder remains unknown. Additional work in these areas is needed to further characterize the nature of endocrine-paracrine dysfunction in affected stallions and to permit the development of effective therapies.

HORMONAL TESTING OF SUBFERTILE AND INFERTILE STALLIONS

The clinician's approach to diagnosis in cases of subfertility and infertility should include a thorough history and evaluation of breeding records over the last few years if possible, a detailed general physical examination, a complete breeding soundness evaluation, and possibly testicular biopsy. An evaluation of the hormonal status of stallions experiencing fertility problems can give insight into the origin and seriousness of the disorder. Serial evaluations over the course of several months and subsequent breeding seasons can assist the clinician in monitoring the stallion for progression of fertility problems. Considerable variation across laboratories and studies exist in the reported normal values of reproductive hormones. For this reason the clinician is directed to interpret results of hormonal testing with their specific laboratory's normal expected values in mind. Readers are reminded that season affects hormone levels in stallions; levels of LH, FSH, and testosterone are lowest in the winter months and highest during the spring and summer. Time of the day also influences results. Hormone levels are lowest early in the day and peak about midday. It has been suggested that the optimal time for hormonal testing in stallions is before 9:00 AM in order to avoid confusion between challenge test responsiveness and normal midday peaks. Recommended hormone tests in stallions include those discussed in the following paragraphs.

Baseline Plasma or Serum FSH, LH, Estradiol, Testosterone, and Inhibin

Single blood samples are taken each morning for 3 consecutive days, or serial samples are taken at 30-minute intervals one morning for a total of six to eight samples. Owing to the pulsatile nature of hormonal secretion, frequent sampling gives a more accurate picture of true baseline levels. Because single samples can vary as much as threefold or fourfold from one another, the mean value of several samples results in a more accurate baseline value. The typical pattern of hormonal aberration observed in stallions with poor fertility includes high FSH levels, low estradiol and inhibin levels, normal to low LH, and normal to low testosterone levels. Hormonal aberrations generally tend to appear in the preceding order as fertility declines over time. Monitoring baseline hormone concentrations on a yearly basis is a recommended routine procedure for stallion management.

Single-Pulse GnRH Challenge (Sample for LH and Testosterone)

This test assesses both testicular and pituitary response to GnRH. Blood is collected 1 hour and again 30 minutes prior to an intravenous injection of 25μg GnRH, and at 30-minute intervals post injection for 2 hours (four additional samples). All blood samples are analyzed for testosterone and LH levels. Subfertile and infertile stallions have lower than expected LH and testosterone stimulation in response to the GnRH injection than normal stallions do, indicating either a primary pituitary problem or

a primary testicular problem; this test is not able to differentiate between the two.

Low-Dose Three-Pulse GnRH Challenge (Sample for LH and Testosterone)

This test may be more useful in assessing pituitary responsiveness to GnRH, particularly in stallions with a primary testicular disorder.[6] Blood is collected 1 hour and again 30 minutes prior to the beginning of the test. Three intravenous injections of 5μg GnRH are given at 1-hour intervals. Blood sampling continues every 10 minutes, starting immediately following the first injection, and continuing for 60 minutes following the final injection. A total of two prestimulation samples and 18 poststimulation samples should be collected. Samples are analyzed for LH and testosterone levels. Similar to the single-pulse challenge, subfertile and infertile stallions show lowered hormone response to the GnRH injections.

hCG Stimulation Test (Sample for Testosterone Levels)

Human chorionic gonadotropin (hCG) has distinct LH-like activity in the horse. This test provides information about the ability of the testis to respond to LH stimulation. Blood is taken 1 hour and again 30 minutes prior to an intravenous injection of 10,000 IU of hCG. Sampling is repeated immediately following injection and at 30-minute intervals for 3 hours following the injection. Samples are analyzed for testosterone. Some workers also suggest evaluating these samples for estrogen levels. Similar to the other challenge tests, subfertile and infertile stallions demonstrate lowered testicular responsiveness to the hCG injection, resulting in lower testosterone and estrogen levels post injection compared to normal stallions.

By compiling the information gathered during thorough physical, reproductive, and hormonal evaluation of breeding stallions, the clinician is better able to inform the owner about a stallion's fertility status and recommend management changes that may improve results. It currently appears that the best treatment for subfertility is good stallion management. Optimum timing of insemination or natural cover, limiting a stallion's book of mares, proper semen handling, and good nutrition and exercise are the best options a clinician can give the owner. Many hormonal therapies have been tried, including GnRH, hCG, and FSH, but all have met with limited and controversial success. In stallions exhibiting severe hormonal changes, the prognosis is guarded to poor. It has been suggested that low estrogen levels (below 124 pg/ml) likely reflect irreversible damage to the seminiferous epithelium, and hormonal therapy in these stallions is likely to be unrewarding.[27]

PROGESTAGEN TREATMENT OF BREEDING STALLIONS

A demand exists for a temporary, reliable, and reversible method of removing undesirable sexual and aggressive behavior common to breeding stallions. Show, competition, and racing stallions may improve their performance if less distracted from their work. Several studies have evaluated the use of progestagens to alter stallion behavior, and their effects appear to be dose-dependent. Miller and associates[28] treated stallions once daily for 30 days with a dose of 0.044 mg/kg altrenogest (Regumate. Intervet Ltd.). These workers demonstrated minimal effects on stallion behavior and no effect of treatment on spermatozoal characteristics. Although plasma FSH concentrations were not affected by treatment with altrenogest, plasma LH, testosterone, estrogens, and inhibin concentrations were all significantly lowered during treatment. Plasma LH, estrogen, and inhibin values returned to pretreatment levels by 30 days after cessation of therapy, but plasma testosterone levels continued to be suppressed even 60 days after cessation of therapy. Johnson and associates (1997)[29] evaluated a higher dose of altrenogest in stallions (0.088 mg/kg) given a 57-day course of therapy. These workers found a significant decrease in scrotal circumference, daily sperm output, plasma testosterone concentrations, libido, and sexual aggressiveness in treated stallions throughout the study, and also demonstrated differences in the testicular morphology of biopsy specimens from treated stallions compared to control stallions. Some of the changes induced by treatment persisted into the 8 week after cessation of therapy observation period in this study as well. Although the higher dose of altrenogest appears to significantly affect sexually aggressive behaviors and sperm output in stallions, the long-term effects and reversibility of this therapy remain unknown and further study is warranted.

PROSPECTS FOR IMMUNOCASTRATION OF STALLIONS

Recently, studies have been aimed at producing a temporarily castrated effect in stallions by immunization against GnRH. Inhibition of reproductive activity by active immunization against GnRH has been shown in several species including the dog, ram, bull, and boar. Initial studies in horses used an oil-based GnRH vaccine that, although effective in producing GnRH antibodies capable of suppressing testicular function, caused significant injection-site reactions in many of the test animals.[30] Use of a water-soluble GnRH vaccine in young colts younger than 2 years of age reduced tissue reactions, and produced reduced sexual aggression and loss of libido in the colts. However, considerable variation between individual animals in immunologic response to the vaccine existed, and multiple injections were required to produce an antibody response.[31] Malmgren and associates (2001)[32] recently evaluated the effect of a five-injection course of a water-soluble GnRH vaccine in three mature stallions. Even though all the stallions responded to the vaccine by production of anti-GnRH antibodies, there were significant variations in response among the individual stallions. Two of the stallions demonstrated a significant reduction in testosterone and estrone sulfate levels following immunization, and the third stallion showed a slight reduction in testosterone and no change in estrone sulfate concentrations. Sexual activity and aggressiveness were markedly decreased in all stallions. Semen quality

deteriorated significantly in two of the stallions by 8 weeks following the initial injection, with significant reductions in total sperm number, sperm motility, and number of morphologically normal sperm being observed. In the third stallion minimal changes in semen quality were seen. The GnRH vaccine appears to significantly reduce sperm production and sexually aggressive behavior in mature stallions, but it does not eliminate stallion-like behavior entirely, and does not produce a sterile effect, which would guarantee contraception. The reversibility of the effect remains unknown. In the early studies, examination of the testes at 9 and 18 months following immunization demonstrated that antibody and testosterone levels had returned to normal; however testicular function had not recovered to preimmunization levels.[30,31] The concept of GnRH immunization to produce a castration effect shows promise, but the reasons for considerable individual variation in response to the vaccine and assurances about its complete reversibility need further study.

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CHAPTER 2

Reproductive Examination of the Stallion: Evaluation of Potential Breeding Soundness

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The stallion breeding soundness evaluation (BSE) is intended to *estimate* a stallion's reproductive potential, which is the current ability of a stallion to impregnate mares, resulting in the birth of a normal foal. This *estimation* is based on criteria provided by the Society for Theriogenology that list reproductive characteristics, including breeding history, physical examination of the whole stallion, physical examination of the reproductive tract, breeding behavior, determination of bacterial growth in association with the reproductive organs, and semen quality measurements. It is recommended that the manual published by the Society for Theriogenology be used as a reference for performing this evaluation.

Following the description and listing of the foregoing characteristics, the clinician categorizes the stallion into one of three classifications: Satisfactory, Questionable, and Unsatisfactory. If a stallion is considered deficient in two or more of these categories (total scrotal width, progressively motility, percent morphologically normal sperm), he is considered either a Questionable or Unsatisfactory prospective breeder. The difference between these two categories is a matter of degree. A stallion considered Questionable would fall *slightly below* the cutoffs for Satisfactory, and an Unsatisfactory stallion would be *very low* in two or more categories. Although this may seem like a subjective judgment, in practice the distinction is usually quite clear.

Historically, a Satisfactory classification is intended to recognize a stallion's ability to impregnate a full book of mares (45 by natural cover or 125 mares by artificial breeding) in an efficient manner (75% of the book in two or fewer normal estrous cycles) using fresh semen deposited in the uterus by natural cover or artificial insemination. These numbers were not based on biologic or intrinsic stallion fertility, but rather on a legal standard (i.e., the number of shareholders) set by the syndicate members. Since the publication of the Society manual, conditions under which stallions are bred have changed, including an increase in the mare book as well as how mares are bred (cooled-stored and frozen semen). The Thoroughbred industry, which uses natural (live) cover exclusively, has increased the maximum number of mares that stallions will breed to over 100, with some stallions approaching 200 mares. The major change in the Standardbred and Quarterhorse industries has been the widespread application of cooled-shipped semen to the point that some stallions breed almost their entire book using this technology, with some stallions breeding 150 to 200 mares.

Since the increase in the number of mares in some stallion books it has become clear that stallions have the biologic potential to breed larger mare books at a level of fertility that is similar to the smaller books described in the Manual. The Thoroughbreds have been using these larger mare books for approximately 10 years. It is the author's opinion that these larger books have not altered the overall fertility and that stallions that would have been Satisfactory prospective breeders for 45 mares are just as capable of handling larger mare books with similar pregnancy rates.

The intent of the classification system is to provide a *framework* around which the clinician can describe, evaluate, and interpret the breeding potential of a stallion. This approach is distinct from the bull BSE, in which a classification less than Satisfactory in many cases results in the elimination of the bull from the breeding population. The primary criteria used are based on sperm quality (motility and morphology), total sperm numbers, and testicular health (testes' size and efficiency of sperm production). Any attempt to define in absolute terms (i.e., using cutoff values), based solely on sperm values and testicular size, a process as complex as fertility will be subject to criticism. However, without guidelines and a framework there is essentially no process by which the results can be interpreted. The guidelines set by the Society for Theriogenology provide a reliable, conservative (i.e., some "fertile" horses may be Questionable, but it is less likely that a Satisfactory stallion will be "subfertile") estimate of a stallion's fertility. For instance, criticism may arise when a young stallion, recently retired from training, is classified as Questionable or Unsatisfactory, but subsequently "does fine in the breeding shed." Once the stresses of racing are removed and the stallion is allowed to "let down," they can show dramatic improvement in their reproductive parameters. In this case, although the BSE may be criticized for "not getting it right," the criteria applied to the day of the evaluation determined that at that point in time the stallion may be limited in his reproductive ability. While the chances of improvement should be discussed with the owner, the final classification must be made based on the findings the day of the evaluation. In addition, the classification allows the

clinician an opportunity to describe management options to the breeder that will maximize the stallion's reproductive potential. As the number of mares bred to stallions increases, the role of management and the input that the clinician can provide will play a greater role.

It is important that the clinician performing the BSE provide more than a simple classification of a stallion, especially in cases when a stallion does not fit into the Satisfactory category. In this case, the stallion owner may become particularly concerned about the reduced classification status of the stallion and assume that the stallion is infertile, subfertile, or that the "condition" is irreversible. It is therefore critical that the clinician be able to communicate, in written form, the significance and interpretation of the findings recorded on the BSE form. It should be recognized that the results of the BSE and subsequent classification of a stallion can have important economic implications for a stallion's breeding future; therefore, the routine evaluation should be performed as completely as possible and thoroughly interpreted to the stallion owner. If a complete evaluation cannot be accomplished, as occurs in many cases, the limitations of the clinician's evaluation should be stated clearly to the owner.

HISTORY

A general as well as reproductive history of the stallion should be recorded as part of the BSE. The general history should include any illnesses and medications administered as well as recent use of the stallion such as in racing or performance. The reproductive history should include a thorough review of the breeding records if the stallion has a breeding history. These records provide an objective record of a stallion's reproductive past, and values such as seasonal pregnancy rate, cycles per pregnancy, and the type of mares in the book (maiden, foaling, barren) can be determined. In general, a stallion's reproductive history falls in one of three categories:

1. *Novice or sexually inexperienced* tend to be young (<3–6 years old) and have recently been in training. It is important to recognize these stallions may not be sexually mature at the time of the evaluation, may have recently been exposed to the stresses of training (exercise and medication), and have not been previously bred to mares. Combined, these factors make these individuals a challenging group to evaluate. Stallions that are not sexually mature or have been under prolonged stress may not produce either the quality of normal sperm or the number of sperm they will produce when sexually mature and removed from the stresses of training. In addition, stallions with no prior breeding experience have no fertility track record to which the results of the BSE can be compared, and therefore, all conclusions about the stallion's future breeding potential must be based on the results of the evaluation alone.
2. *Breeding stallions of any age that are presented with no history of subfertility* may be presented for prepurchase or routine evaluation prior to the breeding season. They are different from novice stallions because they have a previous breeding history and their fertility, in most cases, has been acceptable to the owner. Nevertheless, the clinician should confirm that the fertility reported by the owner is consistent with the recorded history. These stallions have previously sired foals and have an established record of impregnating mares; therefore, the challenge to the clinician is to determine not whether a stallion can get *a mare* pregnant but how many mares he can impregnate in a given time period if the owner wants to increase the number of mares to which he is bred. In addition, the clinician will need to determine, for either the owner or potential buyer, whether the results of the BSE are consistent with the historical fertility of that particular stallion.
3. *Breeding stallions of any age, with a history of subfertility* are presented because their fertility is reduced below a level that is acceptable to the stallion's owner. In some cases, it is incorrectly assumed, by the owner and in many cases the clinician, that the stallion is the sole and primary cause of the reduced fertility. Often the mares to which the stallion is bred and how these mares are bred may be the primary reasons for reduced fertility; therefore, a thorough evaluation of the mare book and management procedures should be performed. Often, as a stallion's fertility decreases, management and mare factors are involved in the decline as well. The clinician should be particularly aware of management and mare factors being major contributors to a reduction in fertility when semen quality is good to excellent.

Group 3 stallions, along with group 1, are perhaps the most challenging, because the veterinarian must make recommendations with respect to the future management of the stallion. Few stallions are intrinsically sterile, but many experience some form of reduced fertility. Therefore, the evaluation should focus on identifying the specific causes of the reduced fertility such that subsequent recommendations can be based on managing those causes. It is also important to identify the source(s) of reduced fertility so that inappropriate therapies are not instituted that may have no effect or worsen the subfertile condition.

Although stallions are classified following completion of the evaluation as a means of simplifying the process for the client, the veterinarian should be prepared to thoroughly explain why that stallion may fall in a particular category and the significance of that particular category. To the layperson, a categorization less than Satisfactory may be interpreted to mean that the stallion is subfertile or sterile or that the "condition" is permanent. In the case of the young stallion described earlier, the lower classification may be temporary and after a period of time the stallion may show improvement. Therefore, reexamination of a stallion may be recommended.

SEMEN EVALUATION

Historically, the hallmark of the BSE is the evaluation of the semen; however, it is only a part of the examination and should not receive more weight than other parts. The routine evaluation of the semen involves the evaluation of sperm motility, sperm morphology, longevity of sperm motility, and the determination of total sperm numbers. The clinician should make an attempt to collect the semen using an artificial vagina, since this provides the most physiologic, representative sample.

Initial sperm motility in the raw form is the first parameter that should be evaluated immediately following semen collection. This parameter is highly dependent on extrinsic factors such as semen handling technique, environmental temperature, semen extender, semen handling instruments, components of the artificial vagina, and expertise of the examiner. Therefore, although this is an important parameter regarding sperm quality, the clinician should recognize that there are many extrinsic factors that can artificially reduce sperm motility resulting in a reading not representative of the stallion being evaluated. All materials (slides, coverslips, microscope stage, pipettes, and semen containers) that will be involved with the evaluation should be maintained at 35 to 37°C, because the portion of the whole semen sample that is evaluated is very small (10–20 µL). Therefore, temperature extremes can quickly alter motility, resulting in an inaccurate estimation. A phase-contrast microscope with a warming stage, although more expensive, is preferred because of the enhanced resolution, especially when morphologic evaluation is performed. Therefore, it is critical that excellent technique be maintained when performing motility estimates, such that the results are meaningful and representative of the stallion being evaluated.

A total and progressive motility estimate is given to each ejaculate. This is accomplished by placing a drop (approximately 10–20 µl) of semen on a clean glass slide and placing a coverslip on top and scanning multiple areas of the slide before determining the estimate. It is recommended that both raw and extended semen be evaluated to establish consistency and to assure that the extender is good quality. Total motility is the total percentage of sperm that exhibit any type of movement, regardless of the movement quality. Progressive motility is intended to identify those sperm whose quality of motion is better (progressive) and theoretically consistent with sperm that have a better chance of "fertilizing." The definition of progressive is subjective and subject to considerable variation due to evaluation conditions, evaluator experience, and the inherent motion of the sperm themselves. A progressive motility score of 60% has been used as a minimum guideline value. It should be recognized that this minimum sets a high standard and many stallions that are "fertile" will fall below this level.

Longevity of Sperm Motility

This parameter has traditionally been evaluated using raw and extended semen at room temperature (~22°C) and is an approximation of the ability of the sperm to live in the mare's reproductive tract. Recently, with the advent of cooled semen it has become important to determine the ability of sperm to withstand the cooling process, which occurs primarily at 5 to 7°C. There are no strict cutoffs to separate good from bad sperm longevity, but stallions whose sperm *consistently* experience very short longevity at room temperature (i.e., 10% motility after 6 hours in the raw form or after 24 hours in the extended form) or after cooling should be further evaluated to determine whether the problem is characteristic for that stallion or whether conditions can be altered to improve longevity. The same extrinsic factors that affect initial motility will be magnified when longevity is evaluated; therefore, certain insults to the sperm are not detected if only initial motility is evaluated, but may be obvious when the semen is evaluated over time. One factor that plays a prominent role in reducing the longevity of motility is the level of seminal plasma in the extended sample. Seminal plasma provides nutrients and ingredients that activate sperm motility, but it does not have the ability to enhance longevity. If longevity is to be measured critically, seminal plasma level should be limited to 5 to 10% of the extended volume.

Morphologic Evaluation

Evaluation of sperm morphologic features can be accomplished by mixing raw semen (several drops if the ejaculate is concentrated or 1–2 ml if dilute) with a buffered-formol saline (BFS) solution. A phase-contrast microscope should be used for this evaluation if BFS is used as a fixative. An alternative is the use of a background stain such as eosin-nigrosin. The use of the BFS has the advantage of higher quality image without producing the artifactual changes that the eosin-nigrosin stain can if not maintained properly. In the stallion, there are specific abnormalities that, when they occur together in high levels, are associated with a reduction in fertility; therefore, individual abnormalities (abnormal heads, detached heads, abnormal midpieces, etc.) should be counted in addition to the number of normal sperm. If an individual sperm has more than one abnormality, both abnormalities should be recorded; however, only one sperm should be counted as part of the total number of sperm that are counted. Documentation of all abnormalities is important to aid the diagnosis and prognosis. Fertility-limiting abnormalities include abnormal heads, abnormal midpieces, detached heads, coiled midpieces, and premature germ cells. In contrast, high levels (40–60%) of individual abnormalities that result in a low percentage of normal sperm are less likely to have a dramatic effect on fertility as a combination of defects. Therefore, using other classification systems, such as identification of primary, secondary, and tertiary abnormalities or identifying only the most proximal abnormality on a sperm may limit the clinician's ability to interpret the results of the morphologic evaluation.

Total Sperm Numbers (Concentration × Volume)

The calculation of total sperm numbers is required to determine the following:

1. Number of mares that can be inseminated from a single ejaculate
2. Efficiency of sperm production based on the total testicular volume
3. Daily sperm output (DSO)

The first step in determining total sperm numbers is calculating the concentration of the sperm sample and requires taking a fixed volume of raw semen and mixing with a fixed volume of diluent. This can be performed using a hemacytometer, spectrophotometer, densitometer, or a computer-assisted motility analyzer. The hemacytometer has the advantage of being the cheapest and the only method that directly counts sperm, because the spectrophotometer and densitometer evaluate fluid opacity and have the potential to give false high readings due to the presence of debris or other nonsperm matter. This is important when some dilute ($<50 \times 106$ sperm/ml) semen samples are evaluated. In this case the contribution of nonsperm material can account for a large percentage of the spectrophotometer or densitometer reading and resulting perception of greater sperm numbers than are actually present, leading to the insemination of a below threshold number of sperm and potentially reduced fertility. These methods tend to work well with ejaculates of higher concentration, in which the debris contribution is less.

Daily Sperm Output

Daily sperm output (DSO) refers to the total number of sperm that a stallion can ejaculate on a daily basis following depletion of extragonadal sperm reserves. These reserves are primarily in the tail of the epididymis and can be three to four times the actual DSO value. Therefore, unless these sperm reserves are reduced prior to making an evaluation of a stallion's fertility potential, the number of sperm collected will not be representative of what will be produced during an active breeding schedule. DSO is a reference point to allow estimation of the number of mares a stallion can breed on a daily basis during the breeding season. Therefore, estimation of sperm numbers based on a single semen sample collected at sexual rest is of limited value.

DSO can be determined by ejaculating the stallion once or twice daily for 5 to 7 days. The first 1 to 5 days either the stallion can breed the mare, which may be more convenient and cost effective for the owner, or his semen can be collected in an artificial vagina and daily semen evaluations can be performed. At the very least, the semen should be collected by artificial vagina during the fifth through the seventh days so those ejaculates can be completely evaluated and the DSO estimated. In general, most stallions reach DSO around day 3 or 4, but this can only be determined retrospectively. It is desirable if the clinician can evaluate as many of the ejaculates as possible so that a more accurate overall impression of the stallion's semen quality can be determined.

The major limitation of DSO is the time and client expense involved in taking multiple ejaculates to establish a plateau of sperm output that is representative of DSO. It can require between three and seven daily semen collections to establish the level of DSO. In the event that the clinician is unable to establish DSO, the alternative is to collect two ejaculates an hour apart and use the sperm numbers in the second ejaculate as an estimate of DSO. Although this has the advantage of convenience and reduced cost, there may be more variation in this estimation than there might be when a DSO value is determined, requiring a broader clinical interpretation.

Once the stallion's total sperm numbers at DSO have been determined, the number of mares to which that stallion can be bred on a daily basis can be determined.

Spermatogenic Efficiency

Determining the total number of sperm as well as the quality of the sperm forms a critical aspect of the breeding soundness evaluation. An additional parameter that can be evaluated is the efficiency of sperm production by the testicles. The intent of this measure is to determine whether, on a per gram basis, a stallion's testes are producing a normal level of sperm. The average stallion should produce 15 million to 20 million sperm per gram of testicular parenchyma. This requires determining DSO, and then comparing those *actual* sperm numbers with the *expected* number of sperm that the testicles should produce based on their size (volume). In effect, this comparison determines whether the testes are producing, in terms of actual numbers of sperm, what they should be, based on the size. Because testicular size is positively correlated with sperm number, larger testes should produce more total sperm, but the efficiency in terms of sperm per gram of testis of a normal testis, of any size, should be similar.

Diagnostically, the actual DSO estimate can be compared to the expected DSO value extrapolated from the volume of the testes. This can be performed in the following sequence:

1. Measure the length, width, and height of each testicle
2. Determine the volume of each testicle using the formula: testicular volume (cm^3) = $0.523 \times$ length $\times$ width $\times$ height
3. Add the volumes of the left and right testes to calculate total testicular volume
4. Calculate expected DSO (billions of sperm) based on the following formula: expected DSO = $0.024 \times$ (total testicular volume in cm^3) $- 1.26$

If *actual* DSO value is less than the *expected* estimate, this indicates that the testes are maintaining their size but the efficiency of sperm production (sperm produced per gram testicular parenchyma) has decreased. This is an early sign of an insult or stresses to the testes that may or may not lead to gross end-stage testicular changes such as a reduction in testicular size or change in testicular texture and may be one of the earliest signs of testicular stress. Another application is when the "small" size of a stallion's testes are questioned. Often "smallness" is associated with "abnormal," but some testes are "small" but function efficiently and normally and therefore may be as efficient as a "larger" testis.

PHYSICAL EXAMINATION OF THE REPRODUCTIVE TRACT

Although the routine BSE focuses on evaluation of the reproductive tract, the clinician must also evaluate the overall physical condition of the stallion. Conditions not related to the reproductive organs can have direct manifestations on reproductive behavior, such as the effect of musculoskeletal pain on the ability of a stallion to comfortably mount and complete the breeding process.

The physical examination of the stallion reproductive tract includes evaluation of the penis and scrotal contents as well as per rectum examination of the accessory glands and ampullae, terminal aorta, bladder, and internal inguinal rings. Ultrasonographic and manual evaluation of these structures can be used to add completeness to the evaluation, especially if the cause of a stallion's problem could be related to these structures or the surrounding areas. The clinician should always be aware of the difficulties associated with per rectum evaluation of either the mare or stallion and only proceed if the evaluation can be performed safely.

The accessory glands of the stallion include the seminal vesicles, prostate, and bulbourethral glands. The ampullae are the terminal portion of the ductus deferens and have a prominent glandular component. Both the seminal vesicles and ampullae are the sites most commonly affected by pathologic changes.

Seminal vesiculitis, uncommon in overall frequency, is the most common condition affecting this gland. It may be uni- or bilateral and is manifest by the presence of polymorphonuclear cells and blood in the final portion of the ejaculate. The extent of blood and PMNs can vary considerably, ranging from only a trace to large amounts of both. Examination findings will vary depending on the severity of the condition and range from normal to palpable changes such as thickening of the vesicle wall and the presence of ultrasonographically detectable echogenic material in the lumen. This condition is commonly misdiagnosed if bacteria originating from the surface of the penis contaminate urethral or semen cultures and it is assumed the bacteria originate from the seminal vesicles. The colonization of bacterial pathogens, such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* on the external surface of the penis is more common than actual infection of the seminal vesicles.

Seminal vesiculitis can be difficult to treat using a systemic regimen of antibiotic therapy because of altered or reduced antibiotic efficacy in the vesicle lumen due to the alkaline environment or the inability of the antibiotic to penetrate into the vesicle wall due to the inflammation. If possible, the vesicle(s) should be treated locally. Local treatment can be performed by passing an endoscope guided catheter up the urethra, into the opening of the seminal vesicles, lavaging the vesicular lumen, then following that with an infusion of an appropriate antibiotic.

Perhaps the most common condition affecting the reproductive status of the stallion is accumulation of sperm in the ampullae, resulting in partial or complete blockage of one or both ampullae. This commonly occurs early in the breeding season because the stallion has been sexually abstinent since the previous breeding season and sperm have had the chance to accumulate in the ampullae. A common presentation is *incomplete* obstruction of the ampullae, resulting in the ejaculation of large numbers (20 billion to 70 billion sperm) of immotile sperm with or without a high percentage of detached heads. Both the immotile sperm and detached heads are a result of the sperm residing at body temperature (i.e., in the ampullae) for time. Treatment for this condition includes per rectum massage of the ampullae, oxytocin administration to contract the ampullar smooth muscle, followed by frequent ejaculation to remove the accumulated sperm and promote the flow of normal sperm from the tail of the epididymis.

This is an important condition and because of the severe presentation (azoospermia or poor sperm quality), it may be assumed that the stallion has an irreversible fertility-limiting condition. In most cases, however, this condition is completely reversible and if diagnosed correctly the affected stallions can be returned to a normal breeding schedule.

Evaluation of the scrotal contents includes manual and ultrasonographic evaluation of the scrotum and testes. Testicular evaluation also includes measurement of the length, width, and height of each testicle by either caliper or ultrasound. Ultrasonographic evaluation is preferred because the testicles can be directly visualized for measurement and qualitative evaluation can be performed on the testicular parenchyma, spermatic cord, and epididymis. The measures used to calculate the volume of each testis as described earlier are subsequently used to determine the efficiency of sperm production. Total scrotal width should also be determined because a value of at least 8.0 cm is one of the criteria considered when final classification is determined.

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CHAPTER 3

Infertility and Diseases of the Reproductive Tract of Stallions

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Although horse breeding has evolved considerably in the last 50 years, reproductive research has not approached the levels that already exist in other species. Much of the research effort in horses has been directed toward understanding stallion fertility. Many factors have been reported to affect a stallion's fertility, including a variety of diseases. Clinicians should have a working knowledge of reproductive physiology, ideal stallion characteristics, management expectations, extrinsic and intrinsic influences on fertility, and methods for semen evaluation before assessing stallion fertility. This chapter deals with some of the diseases and problems that affect stallion fertility.

An evaluation should estimate the stallion's fertility when compared with other stallions with known fertility. Stallions should be expected to achieve at least a 75% pregnancy rate when bred naturally to 40 mares or 90% pregnancy rate when bred artificially to 120 mares. These numbers coincide with a single breeding season, good management, and mares of good fertility.

Stallions can be used for performance or for breeding and are sometimes utilized for both during the breeding season. Commonly, young stallions are retired for breeding after a successful performance career. Stallions may be able to breed and perform during the same breeding season, but physically demanding athleticism adversely affects sperm production.

Stallions are usually evaluated for breeding soundness under specific circumstances: if semen is to be frozen for later use or cooled for immediate transport; if the breeding method should change; prior to purchase; due to an increase in the size of the mare book for the coming breeding season; following a significant injury or systemic disease episode; or when decreased fertility is suspected. Serial evaluations to determine daily sperm output are most helpful following disease or injury or with an increase in the mare book. Following an evaluation, the stallion should be classified as satisfactory, questionable, or unsatisfactory for the intended use.

The farm manager or owner is responsible for daily operation of the breeding farm. He or she determines the degree of veterinary involvement, the quality and quantity of mares in the stallion's book, the methods used to select mares for breeding each day, and the level of herd health practices used. Management decides when to begin and end the breeding season and the breeding method appropriate for the farm. Management skills ultimately determine the farm's success as measured by the number of the stallion's mares that foal the next year. Additionally, management should keep accurate records of the breeding season events. These records are necessary for periodic and end-of-season analysis of breeding efficiency and management expertise. Analysis of the records allows future changes in the breeding operation and identification of problems.

An increased number of barren or problem mares in a stallion's book can make the stallion appear subfertile as judged by conception rates per estrus or foaling rates. Overbooking a stallion, that is, breeding too many mares per unit of time (day, week, or month) could deplete his sperm supply. Overuse may also cause abnormal behavior, especially in immature stallions. Examples of undesirable behavior include reduced libido, aversion to serving an artificial vagina, savaging mares, and aggression.

Breeding farm managers should be knowledgeable about basic reproductive physiology. They should be aware that horses are seasonal breeders and are most fertile during the long days of summer. Practically, this knowledge could potentially reduce the number of breedings per estrus and per pregnancy. Management must utilize veterinary expertise to enhance breeding efficiency.

Inappropriate herd health measures or poor breeding hygiene may adversely affect a stallion's fertility. Spermicidal chemical residues on equipment that contact the stallion's ejaculate may cause failure to conceive. Veterinary clinicians asked to help find causes for infertility should carefully examine the management practices used on the breeding farm as a potential source of the stallion's perceived subfertility.

The age at which puberty occurs can vary among breeds of horses, but stallions generally begin producing significant spermatozoa for ejaculation at an average of 83 (±2.9) weeks of age. Sexual maturity may not occur for another 1 to 3 years, depending on breed.

Extremes of body condition can affect fertility. Stallions that are maintained either too fat or too thin may experience decreased sperm production when compared with stallions in optimal body condition. Adjustments in the plane of nutrition are necessary during certain times of the year to maintain optimal body condition; stallions not actively breeding mares require lower caloric intake, as opposed to during the breeding season when they need more calories. The greater the number of breedings per week and the more active a stallion is when not

breeding, the more calories he will need. Ideally, periodic nutritional adjustments should be made to maintain stallions in optimal body condition.

The greater the frequency of ejaculation, the more the stallion's sperm numbers may be depleted. Younger stallions with smaller testes produce fewer spermatozoa, which can be depleted more quickly, than do mature stallions with larger testes. The optimal number of ejaculates per unit of time depends on information found by serial semen collections, as well as on the stallion's age and testicular size, the season of the year, and appreciation of the stallion's inherent fertility gained by assessing his previous ability to impregnate mares.

Testicular volume is a relatively accurate predictor of daily sperm output (DSO). Based on a formula for determining the volume of an ellipsoid, each testicle can be measured (mm) by ultrasonography[1] (see Chapter 2). This estimate may be used to compare with actual numbers produced by routine semen collection or with breeding suitability examination results. It may also serve as a basis from which a stallion's book may be predicted. A negative deviation from the expected DSO warrants further investigation for potential causes.

Several breeding methods or schemes are recognized for stallions: natural service either in a pasture setting or controlled by in-hand breeding; artificial insemination; cooled semen shipment to mares located at a farm other than where the stallion resides; and collection of a stallion's ejaculate for cryopreservation and storage for later insemination. The choice of breeding method may be dictated by the breed registry or association, governed by specific rules from such, or left entirely to the choice of the farm management. There are advantages and disadvantages to each of the breeding methods.

NONINFECTIOUS CAUSES OF SUBFERTILITY

Diseases that may cause infertility in a stallion can be arbitrarily divided into noninfectious and infectious sources. Either can affect the stallion's ability to produce or deliver sufficient normal spermatozoa to effectively produce pregnancy in mares. These disease problems may affect only the reproductive tract or may be systemic. Infectious problems may be caused by bacteria or viruses that can be transmitted venereally, by direct contact between horses, or by fomites. Noninfectious problems are not transmissible but can influence normal spermatozoa production and pregnancy rates of the afflicted stallion.

Physical Trauma

Stallions that must perform natural service will be at risk of trauma caused by the mare or from events associated with breeding. Poor footing in the breeding area may lead to musculoskeletal injuries that can limit the stallion's mounting ability or result in psychological trauma or both. When a mare kicks a stallion during breeding, there may be damage to his external genitalia, resulting in swelling, edema, abrasions, or lacerations. Blunt trauma to the penis and sheath may cause enough swelling to prevent retraction of the penis into the sheath (paraphimosis). With the penis swinging free outside the sheath, normal blood circulation to the glans is inhibited, leading to further swelling and edema. In addition, abrasion of the penis will occur as the stallion moves about. Abrasions can rapidly progress to ulceration, and without aggressive therapy the stallion's penis may require amputation because of irreversible damage.

The initial treatment consideration in the case of trauma should be stall confinement to prevent further damage. Second, the penis should be replaced into the stallion's sheath. With minimal swelling, the penis may be easy to replace. When edema is extensive, the swelling should be reduced before replacement within the sheath is attempted. Application of a compression bandage beginning at the glans penis may decrease swelling sufficiently for replacement. With the penis inside the sheath, nonabsorbable sutures should be placed across the sheath opening. Retaining the penis in the sheath will allow return of normal blood circulation, reduce edema, and prevent further damage. The stallion can urinate without extending the penis. Urine scalding of the internal sheath may result, but its effect may be lessened with daily application of emollients.

If penile swelling cannot be reduced or the penis prolapses after the sheath has been sutured, then a sling should be fashioned to support the penis.[2] A sling can be fashioned from a bag used for laundering delicate fabrics. These bags are made from soft cotton material and provide support without damage to the penile tissues. The bag should be cut into a rectangle of the proper length and width to accommodate the penis. The penis is suspended in the soft cloth bag close to the ventral abdomen. The bag is held in place with 3-inch gauze straps attached to the corners and tied over the stallion's croup. Several thicknesses of cotton beneath the gauze straps are necessary to prevent skin injury.

An alternative technique has been described using a plastic bottle that has been modified to be used as a preputial "splint." A 1-liter saline bottle has the bottom cut out and the sharp edges padded with tape to prevent abrasions. The bottle is lubricated with petrolatum jelly and the opened bottom is inserted over the end of the penis, once it is reduced to a size that will fit inside the bottle. The neck of the bottle is used to anchor stint supports of long 2- or 3-inch gauze. Four stints are made, two pair to go laterally over the flank to the croup, and two to go caudally on either side of the prepuce and testicles and up between the legs skirting the tail-head to meet the lateral stints over the back, and are tied. The bottle top is left open for urine to be passed through. The splint is reset daily following hydrotherapy as needed and the penis monitored closely for any sign of abrasion caused by the retention device.

With the penis either supported as described or placed within the sheath, cold hydrotherapy should be provided for 20- to 30-minute intervals, two to four times daily. Such therapy may enhance local blood circulation, which decreases swelling and edema. Hydrotherapy should be

continued as long as swelling and edema persist. Systemic administration of diuretics, glucocorticoids, and nonsteroidal anti-inflammatory drugs may be considered for reduction of edema but will not supplant the need for hydrotherapy and penile support. With full-thickness abrasion of the penile skin, systemic antibiotics are indicated to reduce bacterial contamination because secondary infections delay healing and promote swelling and edema.

Priapism is defined as penile erection in the absence of sexual stimulation that does not recede within a short period of time. The corpus cavernosum penis (CCP) is engorged with blood that soon develops fibrin and undergoes spontaneous clotting. The etiology is unclear but has been associated with the administration of phenothiazine tranquilizers, general anesthesia, neuromuscular dysfunction, equine herpesvirus infection, cachexia, dourine, and idiopathic. Early medical management consists of diuretics, nonsteroidal anti-inflammatory agents, manual massage, cold hydrotherapy, and suspension and support of the penis. General anesthesia and massage of the penis to decrease venous engorgement, application of a compressive elastic bandage, and lavage of the CCP using 10 to 12 gauge ingress and egress cannulas along with heparinized saline have all been described as useful when medical management is unrewarding or the value of the stallion warrants more aggressive therapy. When lavage is performed it is recommended to continue until arterial blood is flushed from the egress cannula. Several attempts to lavage or irrigate the CCP may be required for resolution. More aggressive surgical intervention may be considered by creation of a shunt between the CCP and the corpus spongiosum penis (CSP). Failure to aggressively manage and resolve this condition will lead to loss of the stallion as a breeding animal and may necessitate castration and phallopexy (Bolz technique).[3]

Trauma to the scrotum and testes may occur along with injuries of the penis and sheath. Scrotal and testicular injuries or edema require aggressive therapy to reduce the likelihood of permanent damage to the stallion's fertility due to compromise of the thermoregulatory mechanism. With swelling, edema, or inflammation of the testes and scrotum, abnormal spermatozoa will be produced until normal heat exchange can be restored.

When the scrotum or testes are injured, ultrasonographic examination of the involved organs is beneficial to help determine the extent of damage. Testicular injuries could permit exposure of spermatozoa to the systemic circulation and result in antisperm antibody production. Antisperm antibodies can cause subfertility or sterility.

Stallions that experience genital injuries may develop psychological or behavioral abnormalities, scarring within the reproductive system, and immunologic changes as described. Such residual damage may not become apparent until the stallion is reintroduced to the breeding shed routine. Management should be cautioned about the likelihood of psychological or behavioral problems, or both, that may occur in injured stallions and should be encouraged to slowly and gradually introduce the stallion back into his former routine.

Hemospermia

Hemospermia causes infertility that may vary with the quantity of red blood cells in the ejaculate. The mechanism by which blood causes infertility has not been determined. Hemospermia may result from either external or internal lesions in the stallion's reproductive organs. External lesions include those from traumatic injury such as lacerations, hematomas, abrasions, ulcerations, and dermatologic conditions. Lacerations and abrasions may occur during breeding, as when the mare's tail hairs cut the glans or urethral process during coitus. Hematomas may rupture externally or into the urethra causing spontaneous bleeding or hemorrhage during urination or ejaculation. Ulcerations may arise from infectious disease lesions either externally or internally.

Internal urethral ulcerations lead to contamination of the ejaculate with blood because of rapid expansion and contraction of the tissues during urethral pulsations. The ulcers may be located anywhere from the urethral process to the pelvic urethra near the ejaculatory ducts. Spontaneous hemospermia can occur in stallions that are bred heavily, as a sequela to bacterial urethritis, from scar tissue that results from a stallion ring, or from infection of the accessory sex glands.

Diagnosis of hemospermia may begin by observation of accumulated dried or fresh blood on the stallion's rear legs, sheath, or ventral abdomen. Fresh blood may be noted on the stallion's penis following coitus or may be visible grossly in the collection container after semen collection. If fresh blood appears on the stallion's penis following coitus, the source of hemorrhage could be from the mare's genitalia (vaginal or hymen rupture, or trauma of a varicocele within the vestibule) or from the stallion. To determine the origin of blood, the mare must first be ruled out as a source, then the stallion's penis and sheath should be examined with a small-diameter flexible endoscope, with care taken to prevent iatrogenic urethral trauma. The examiner should be cognizant that ulcers may occur anywhere along the urethra. The most frequent location for urethral ulcers may be in its pelvic portion as the urethra bends distally over the ischial arch, or near the ejaculatory ducts at the cranial termination of the urethra.

Treatment for hemospermia consists of sexual rest, local medications placed in the pelvic urethra via a perineal urethrostomy, systemic antimicrobial administration, acidification of the urine, or surgery (perineal urethrostomy). Laser surgery may be useful for cautery of the ulcer(s).

Dermatologic Conditions

Invasive dermatologic conditions, such as habronemiasis, lead to ulcers and can be found on the urethral process, glans, or prepuce. The ulcerated area can hemorrhage or cause pain during ejaculation. Recommended therapy usually consists of a combination of topical medications designed to kill the *Habronema* larvae and protect the mucosa, sexual rest to allow time for healing, systemic anthelmintics or glucocorticoids or both, or cautery of

the lesions. Surgical excision of the urethral process has been suggested but may result in stricture of the urethra or hemospermia. Complete healing may not occur until the offending stable flies disappear with onset of winter. Some horses seem prone to have chronic recurrence of this disease, regardless of therapy.

Tumors of the stallion's external genitalia cause subfertility by preventing erection, obstructing the urethra, causing hemorrhage into the ejaculate, or causing pain during ejaculation. Some tumors, owing to their size, may prevent copulation or normal retraction of the penis into the sheath.

Superficial neoplastic lesions must be differentiated from dermatologic conditions such as habronemiasis. Both may appear granulomatous and ulcerative and are associated with hemospermia or pain during breeding. A biopsy of the lesion should be obtained and analyzed prior to initiating therapy. Histopathology results can aid in proper therapy choices and accurate prognosis for return to breeding. Squamous cell carcinoma (SCC) and sarcoid are the most common external genital tumors and most frequently occur in stallions with some nonpigmented skin on the penis or prepuce. Successful treatment of tumors depends on early diagnosis and aggressive therapy. Neoplasms may be surgically reduced in size or completely excised. Initially, it may be appropriate to reduce the size of the tumor and then treat with cryosurgery, hyperthermia, or immune stimulation. Intralesional injection of cisplatin has been described for penile SCC, squamous papillomas, and sarcoids. Topical application of 5-fluorouracil in cases of SCC has been reported to be successful in causing tumor regression. It is necessary to provide sexual rest until the lesions have healed sufficiently to prevent hemospermia. During the breeding season, it may be necessary to assign the stallion's mare book to another stallion.

Tumors of the testes appear less frequently than skin tumors. Seminoma, the most common testicular tumor, may be either benign or malignant, and a successful outcome depends on early identification and treatment. Equine seminomas may develop from exposure of spermatozoa or seminal fluid to the surrounding tissues. A seminoma may begin as unilateral hardening and enlargement of a testis with subsequent invasion of the epididymis and tissues surrounding the spermatic cord and extension into the abdomen along the cord or lymphatics. The course of neoplastic invasion may be slowed or eliminated with hemicastration and removal of visibly diseased tissue. Once neoplastic invasion of the lymphatics has begun, the stallion may survive only a few months.

Urinary Tract Obstruction

Urinary tract obstruction may occur as a result of urinary calculi formation or scarring due to previous injury or surgical intervention. Urinary calculi may cause hematuria, cystitis, or obstructive disease. An accumulation of dried smegma in the urethral diverticulum of stallions may cause pain during urination. For a review of clinical signs, diagnosis, and treatment of urinary calculi, the reader is referred to other published sources. The treatment of smegma accumulation ("bean" formation) usually involves sedation of the stallion and removal of the "bean" by gentle flushing and washing with mild soap solutions.

Inability to Mount and Copulate

Lameness or injury may become related to fertility, especially when it involves rear limb problems, because it may delay or prevent proper mounting and copulation or lead to behavioral problems. Lameness problems are most critical when pasture breeding is used because the stallion must be able to follow his mares and copulate. Other breeding methods may allow more tolerance for lameness problems. Lame stallions required to cover tall mares can be helped if the mare's rear feet are placed in a pit during copulation. Also, stallions whose semen is collected while they mount on a phantom have the greatest flexibility because the phantom can be lowered to a more comfortable height. Careful observation of the stallion's movement and attention to subtle gait changes should allow early diagnosis of lameness and permit aggressive and successful therapy to keep the stallion from missed breedings. Necessary therapy may range from surgical intervention to administration of topical or systemic medications designed to alleviate pain associated with copulation. Stallions may also be trained to collect semen while standing on the ground, avoiding the jump mare or phantom altogether, using an artificial vagina.[4]

Ex copula ejaculation using prostaglandin F_2 alpha, detomidine, xylazine, or imipramine has been described.[5] Reports have included a variety of regimens, dosages, schedules, and combinations. Overall success rates have ranged from 30 to 75% for successful induction of ejaculation. One of the more tested and successful descriptions includes a treatment of 3 to 5 mg/kg imipramine orally, followed in 2 hours with xylazine at 0.66 mg/kg, intravenously. The stallion is in a stall without exposure to a tease mare. A collection device is made from a ring or hoop of wire or plastic large enough to insert snugly into the open arm of a sterile obstetrical sleeve. The hoop or ring is fashioned as a sling over the prepuce with gauze stints that extend laterally over the flanks to the croup and tied; additional stints may pass caudally between the rear legs and around the tail-head to the croup for better stability and anchoring. Tying off above the wrist area can shorten the sleeve and the excess material removed with scissors. The range of time to ejaculation in successful attempts was 1 to 14 minutes after the xylazine was administered. Occasionally, the stallion will ejaculate before the xylazine administration, and many will ejaculate in response to xylazine alone, without the imipramine before treatment.

Many neurologic diseases that affect stallions are discovered during a general physical examination. If such neurologic conditions are found to affect the stallion's ability to mount or copulate, then he should be rejected as a prospective breeder, or considered a candidate for ex copula ejaculation management.

Some diseases that affect a stallion's ability to mount and copulate include equine protozoal myeloencephali-

tis (EPM), cervical vertebral instability, equine herpesvirus myeloencephalopathy, and penile paralysis.

The parasitic disease EPM has been reported to cause cervical spinal cord and brain stem lesions. The *Sarcocystis neurona* organism can affect horses of nearly any age and causes microscopic damage to the ascending and descending spinal tracts, leading to rear limb instability.[6] An antemortem diagnosis of EPM is tentative and complicated by numerous false positive diagnostic test methodologies. A differential diagnosis should include cervical vertebral instability in horses younger than 3 years of age, neuritis of the cauda equina, and other neurologic disorders. Early diagnosis and treatment of EPM with a potentiated sulfonamide or with ponazuril may allow alleviation of clinical signs.

Cervical vertebral instability results from ventral spinal cord compression with proprioception deficit and ataxia of the rear limbs. Diagnosis of this condition depends on observation of clinical signs and on radiography. Generally, this condition is not treated, and, because it is likely to be heritable, afflicted males are not used for breeding.

Myeloencephalopathy induced by equine herpesvirus infection (EHV-1) occurs sporadically, and economic losses may vary.[7] The short-term mortality rate is low, but the long-term effects may be much more significant. Loss of rear limb proprioception, loss of urinary bladder control, inability to achieve erection, and rectal paralysis are potential complications that may prevent an affected stallion from performing his intended function as a breeding horse.

Spinal cord lesions in the ascending or descending spinal tracts, or both, cause variable loss of motor control to the rear legs. Motor control deficits limit ability to mount, thrust, and, perhaps, ejaculate. Affected stallions may learn to compensate for their deficits and with guidance and support from the handler may be capable of normal fertility. These considerations should be reserved for those stallions that develop nongenetic ataxic diseases.

Penile paralysis may result from administration of phenothiazine-derivative tranquilizers, spinal trauma, debilitating diseases, starvation, or direct trauma.[8] The etiologic pathogenesis of phenothiazine-caused penile paralysis remains obscure. It is recommended that administration of phenothiazine-derivative sedatives to breeding stallions be avoided. If sedation becomes necessary, other types of tranquilizers, such as xylazine or detomidine, may be used with less concern over penile paralysis. In the event that tranquilizer-induced paralysis occurs, it should be managed as described previously. Some penile paralysis cases can be resolved with proper care; however, many others will require surgical amputation of the penis (phallectomy).

Psychological Trauma

Stallions that suffer injuries associated with copulation may suffer psychological as well as physical trauma. After recovery from the physical trauma, the stallion may exhibit abnormal breeding behavior such as refusal to mount mares, mounting without intromission, and ejaculation failure. The more serious the physical trauma, the greater the likelihood of psychological damage. However, young timid stallions may exhibit psychological abnormalities from relatively minor injuries. Treatment of stallions exhibiting psychological or behavioral abnormalities requires sufficient time for recovery, as well as extreme patience by management. Psychologically affected stallions should be exposed only to mares at the peak of estrus; these mares must be as receptive and gentle as possible. When a session results in ejaculation and preinjury behavior, the stallion should be properly rewarded and praised and then removed from sexual contact until the next day. The handler should use all types of reward stimuli when the stallion shows the desired behavior. Positive reinforcement will ultimately restore normal sexual behavior. Care should be taken to prevent any subsequent copulation-associated injury; such reinjury would be a negative reinforcement with serious consequences.

Excessive disciplinary measures applied to breeding stallions can cause psychological problems and abnormal behavior. These behavior abnormalities may be manifested as failure to complete copulation or disinterest in mares. Treatment of such cases should proceed as described earlier. Excessive discipline cases often present a difficult diagnostic challenge for the clinician. A tougher challenge is to convince the stallion handler that he or she may be having an adverse effect on the stallion's behavior and thus his resultant fertility.

Urospermia

Urination during ejaculation will likely cause subfertility and may be caused by psychological abnormality or organic disease.[9] If the condition is behavioral, it may be amenable to treatment as outlined for other behavioral conditions. Management may include breeding or collecting semen only after observed urination. Administration of a diuretic followed by placing the stallion in a freshly bedded stall may assist this process. Those cases resulting from loss of bladder sphincter control may be treatable with the administration of flavoxate hydrochloride. Semen can be collected from the afflicted stallion with an open-ended artificial vagina to allow separation of the ejaculate fractions because many affected stallions urinate with the final ejaculatory pulsations.

Endocrine Abnormalities

Control of reproduction involves the gonadal steroids, gonadotropins, and other influential substances (e.g., autocrine and paracrine hormones and factors). These hormones influence the development of sexual organs, display of sexual behavior, production and maturation of spermatozoa, and production of seminal fluids. Endocrine abnormalities or imbalances can dramatically affect fertility.

Currently, measurement of follicle-stimulating hormone (FSH), luteinizing hormone (LH), total estrogens (TE), testosterone, thyroxine (T_4), tri-iodothyronine (T_3), cortisol, inhibin, and insulin in stallions is available.

Imbalances of TE and FSH in stallions can be associated with subfertility.[10] It has been shown that elevated concentrations of FSH and decreased concentrations of inhibin and TE are almost always associated with subfertility and onset of testicular degeneration, as evidenced by an increased number of breedings per pregnancy. Conversely, there have been cases of subfertility without demonstrable endocrine imbalance. One reason postulated for this paradox is the lack of evidence to demonstrate whether an endocrine imbalance precedes or follows seminiferous tubular degeneration. However, other reports indicate that declining inhibin and TE concentrations precede testicular degeneration. Periodic endocrine screening of breeding stallions to aid in predicting the onset of testicular degeneration is recommended.

Circulating concentrations of LH and testosterone should be measured before and after challenge with exogenous gonadotropin-releasing hormone (GnRH). Because circulating endocrine concentrations are affected by the season of the year and normal diurnal changes occur, these variations must be considered in the evaluation. The results of a GnRH challenge test may indicate a relationship between low total estrogens, low inhibin, high FSH concentrations, and subfertility. Stallions with idiopathic testicular dysfunction will display low motility (20–30% progressive motility), and marginal morphology (±40% normal sperm) along with some slight change in testicular consistency. In time, these stallions show decreasing inhibin, increasing FSH, and decreasing estrogen. Peripheral concentrations of testosterone and LH remain normal until such time as the sperm output significantly declines. Recent evidence in the stallion points toward initial pathologic dysfunction at the level of the Sertoli cells, spreading to the germ cells and then the Leydig cells, eventually moving to the hypothalamus and then the pituitary. Testicular inhibin (Sertoli cell) declined prior to changes in testicular steroid hormones in stallions studied progressively from normal fertility to subfertility.

Subfertile stallions may have low testicular response with respect to GnRH challenge tests because of a dysfunctional hypothalamic-pituitary axis producing bio-inactive LH, or a primary testicular dysfunction. Testicular biopsy may serve as an early indicator of endocrine/paracrine/autocrine dysfunction when used diagnostically in stallions being assessed for breeding soundness. The testicular tissues are submitted for direct endocrine analysis.

The efficacy of exogenous gonadotropin for treatment of stallion infertility has been seriously questioned. This lack of efficacy has been blamed on the paucity of knowledge regarding the interplay of hormone concentrations and season in normal and infertile stallions; lack of diagnostic criteria to indicate when treatment becomes necessary; and the lack of efficacious endocrine preparations to be used in stallions.

Other endocrine-related problems may be encountered in stallions. Cortisol, insulin, and glucose play a role in the diagnosis of pituitary tumors. Clinical signs of pituitary adenoma include chronic weight loss, shaggy hair coat with abnormal shedding patterns, polyuria and polydipsia, and hyperglycemia. These signs will likely appear well after age-related onset of testicular degeneration has contributed to fertility problems. Horses presented with evidence of testicular degeneration and one or more of these clinical signs should be tested for depression of endogenous cortisol in response to glucocorticoid administration. Thyroid dysfunction may be suspected in stallions with depressed libido and excessive body weight. Affected animals classically maintain their body condition with minimal caloric intake. Diagnosis of hypothyroidism should be based on T_3 and T_4 assays, thyrotropin-releasing hormone (TRH) challenge, and clinical signs. Therapy for hypothyroidism consists of daily supplementation with sodium levothyroxine (10 mg) or iodinated casein (5 mg). These measures should be combined with dietary restriction and exercise.

INFECTIOUS DISEASES

Systemic Infections

Systemic infectious diseases may decrease fertility by causing reproductive tract inflammation or through their effects on other systems. Such conditions may be readily transmissible through a variety of means, including venereal.

Systemic illness or any condition that causes pyrexia may decrease fertility. Prolonged pyrexia, inflammation of the scrotum, testes, or epididymides, may interfere with the thermoregulatory mechanism of the testes. Compromise of the thermoregulatory mechanism results in a significant reduction of progressively motile spermatozoa, increased morphologic defects, and significant reduction of total sperm. These effects last 60 to 70 days after resolution of the problem. Toxins, chemicals, or other substances may affect fertility by causing systemic illness or by direct effect on the testes. Systemic illnesses that produce lethargy or inappetence may decrease or eliminate libido. Such systemic conditions may not decrease numbers of spermatozoa or render them incapable of fertilization, but when a stallion is unwilling to copulate, his fertility is reduced. Treatment should be aimed at rapid elimination of the primary cause of the illness and should allow resumption of normal sperm production.

The clinician should recommend complete semen evaluation as soon as possible following abatement of the condition and reexamination 60 days later. During the 60-day recovery period, management should alter the stallion's mare book to compensate for his subfertility. After a 60-day rest, an estimate of fertility based on evaluation of semen quality will help determine when the stallion may be able to return to the previous breeding level. If poor semen quality persists, a reduced breeding load must be continued and further evaluations planned at approximately 15-day intervals until semen quality returns to preinjury status. This same protocol can be used for any condition found to temporarily decrease fertility.

Bacteria and Viruses

Subfertility caused by bacteria or viruses may affect the internal or external reproductive organs. These microorganisms may or may not be transmitted venereally. The stallion's external genitalia are not sterile, and most cultures or samples from these sites yield bacteria that may or may not not be related to infertility. Many bacteria have been considered part of the normal flora. Pathogenic bacteria that should be of concern are *Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus zooepidemicus, Taylorella equigenitalis,* and, rarely, others. These bacteria may be transmitted venereally to susceptible mares or exist in the stallion without being transmitted to mares.

Overt clinical signs of bacterial infection of the internal or external genitalia may not be apparent. Rather, infection is suspected based on assessment of breeding results and culture of the reproductive organs. Confirmation of suspected subfertility should begin with an indication that mares have been bred during several cycles without conception; reduced pregnancy rate; an increased incidence of early embryonic deaths; or a combination of these findings.

Once suspicions of subfertility are confirmed, evaluation of the stallion should be performed. In addition to semen collection and evaluation, the genitalia should be cultured. Prior to collecting culture swabs, the genitalia should be rinsed with clear warm water to remove debris and smegma and then dried with a clean cloth or paper towel. Scrubbing with soap, detergent, or disinfectant should be avoided.[11] Cultures should be obtained from the fossa glandis, the shaft of the penis, the urethra following sufficient teasing to cause discharge of preejaculatory fluids, the urethra following ejaculation, and optionally from the raw ejaculate. The recess of the fossa glandis has been shown to be a reservoir for potential pathogens. All swabs should be protected to prevent drying and rapidly transported to a microbiology laboratory using an appropriate transport nutrient medium. Aerobic and microaerophilic culture conditions need to be considered especially when screening for *Taylorella equigenitalis*.

Evaluation of the culture results should include consideration of the anatomic location where bacteria were found, whether they appeared on the growth medium in significant numbers within the first 24 hours of incubation, the species of organisms isolated, and whether the results correlate with subfertility. To be considered pathogens, bacterial colonies should grow in significant numbers within the first 24 hours of incubation, the organism should be uniformly distributed in all parts of the reproductive tract, and management records must indicate abnormal breeding efficiency. There may be an increased number of inflammatory cells in the ejaculate when the internal reproductive organs have been invaded by pathogens. Stained smears of the ejaculate should be examined for inflammatory cells. Isolation of pathogenic organisms from maiden stallions should be considered suspicious and the procedure repeated.

Pathogenic bacteria may be found throughout the stallion's reproductive tract, but the external genitalia and accessory sex glands harbor the organisms most frequently implicated as causes of subfertility. Pathogens residing on the epithelial surfaces of the penis and sheath or in the accessory glands may be transmitted to mares.[12] However, venereal transmission of pathogenic bacteria is difficult to substantiate by uterine cultures obtained immediately after breeding. Increased breeding of contaminated mares by natural service, or iatrogenic alteration of the normal bacterial flora of the penis and prepuce, or both, may be involved in increased bacterial contamination as the breeding season progresses. The first sign of increased bacterial contamination might be evidence of subfertility within a stallion's mare book as the season progresses.

The accessory sex glands contain pathogenic bacteria less frequently than the external genitalia. However, when present, infected accessory glands may exhibit localized swelling with discharge of purulent exudates into the ejaculate. Diagnosis of accessory gland infection can be confirmed by selective cultures, palpation, transrectal ultrasonography, urethroscopy, splitting the ejaculate during semen collection, and examination of stained preparations of the ejaculate for inflammatory cells. Documented accessory gland infections are difficult to ameliorate with systemic antibiotic therapy. Breeding management programs might include breeding with the minimal contamination technique or artificial insemination with extenders containing antibiotics. An ejaculate should be collected and mixed with semen extender containing antibiotics, and sufficient time (30 minutes) should be allowed before insemination. If artificial insemination cannot be used, antibiotic-containing semen extender should be infused into the uterus prior to the mare's copulation with the infected stallion. This method does not entirely prevent bacterial transmission but may allow successful breeding when other options do not exist. Another method for preventing bacterial transmission may be lavage of the mare's uterus 4 to 8 hours after copulation.

Bacterial contamination of stallions may arise from their covering infected mares or from overzealous scrubbing of the external genitals with soaps or detergents or may be transmitted by improperly cleaned equipment (e.g., artificial vagina). The external genitalia of breeding stallions should be cleansed with warm clear water only, and the use of soaps, detergents, and disinfectants should be avoided. Separate semen collection and handling equipment should be designed for each stallion on the farm and maintained in a clean and sterile manner to limit the probability of disease spread.

Taylorella equigenitalis, a bacterium transmitted venereally among horses, has been reported in Europe and has caused several outbreaks in the United States. The organism has been found to persist in the clitoral fossa and clitoral sinuses of carrier mares. Transmission to susceptible stallions during coitus has been reported; contaminated stallions in turn transmit the bacterium to susceptible mares. The organism may also be transmitted by fomites used in handling and treating mares or stallions. Mares infected for the first time usually exhibit a mucopurulent vaginal discharge for about 2 weeks. After 2 weeks, the

discharge disappears without treatment and the mare becomes a chronic carrier. Stallions are asymptomatic carriers and exhibit no apparent reduction in fertility, except when bred to susceptible mares. Stallions continue to transmit bacteria for an unknown time period.

Diagnosis of *T. equigenitalis* in stallions should include cultures of the urethral fossa, urethra, prepuce, the preejaculatory fluid, and semen.[12] Mares exhibiting vulval discharge should have cultures taken from the clitoral fossa and sinuses and the uterus. This organism requires special handling and transport on Amies charcoal medium to ensure proper growth and identification. To assist positive identification of the organism a polymerase chain reaction (PCR) assay has been developed for use in some laboratories. About 2 to 3 weeks following initial exposure, the mare will develop antibodies and be seropositive on a complement fixation test for *T. equigenitalis*. However, such tests are of no benefit for diagnosis of the disease in stallions. The International Organization of Epizootics (Organization International de Epizooties, OIE) recommends treatment to include daily washing of the infected stallion's penis with 2% chlorhexidine scrub, followed by 0.2% nitrofurazone cream over the entire penis and packing the urethral fossa.[13] The treatment is applied once daily for a minimum of 5 successive days.

Two important viral diseases of breeding horses in the United States are equine arteritis virus (EAV) and equine herpesvirus III (EHV-3; coital exanthema). There have been numerous outbreaks of viral arteritis at racetracks and on breeding farms in recent years. Acute EVA infection may cause systemic illness with clinical signs such as fever, stiffened gait, lacrimation and conjunctivitis, nasal discharge, skin rash, and edema of the lower limbs, scrotum, sheath, and head. Abortion has been known to occur in acutely ill or convalescent mares. Acutely infected horses transmit the disease primarily via the respiratory tract.

Stallions may become infected with EAV via aerosol transmission (nasopharyngeal and genitourinary secretions) from acutely ill horses or by venereal transmission from breeding an acutely infected mare. The acutely infected stallion may initially develop scrotal edema, resulting in temporary infertility from disruption of the testicular thermoregulatory mechanism. Compromise of the testes is primarily indicated by decreased progressive motility of sperm while other seminal parameters remain essentially normal. Infected stallions become asymptomatic carriers and shed the virus in the ejaculate. Virus shedding in the ejaculate may last from a few weeks to more than 3 years. Mares bred by chronic carrier stallions usually do not develop acute illness or reduced fertility, but they may transmit the virus venereally to another stallion.

Infection with EAV may be demonstrated in stallions by seroconversion using serum neutralization or complement fixation tests on serum samples collected at 3- to 40-week intervals. Attempts should be made to isolate the virus from the ejaculate of seropositive stallions, or they should be test-mated to seronegative mares. Test-mated mares are then monitored for seroconversion. After infected stallions have been identified, control measures should be instituted to prevent spread of the disease.

Measures for control and prevention of EAV include administration of a modified live vaccine approved for use in nonpregnant mares and in stallions in the United States or a formalin-inactivated vaccine available in Europe. Control programs should include stallions found to be chronic shedders of EAV in the ejaculate, first-season stallions that may be exposed to EAV, and seronegative broodmares. Vaccinated horses or any other horses with titers to EAV may not be eligible for export to certain countries.

Coital exanthema, caused by EHV-3, may occur as an external genital infection in mares and stallions. At times, the virus can cause systemic signs of infection, including dullness, anorexia, and fever. However, the most common evidence of this disease is vesicles on the penis and prepuce of stallions and on the vulva of mares. Stallions can become infected by coitus with infected mares and subsequently transmit the disease venereally to other mares. A few days after exposure, vesicles form on the penis and prepuce, rupture, then become pustules with ulcers beneath crusts. These ulcers may cause pain during coitus or hemorrhage into the ejaculate, or both. The disease is self-limiting and noninfectious once the ulcerative lesions have healed. Depigmented skin areas on the penis, prepuce, and the mare's vulva may follow once the ulcers have healed. Immunity to this virus is relatively short, and previously afflicted horses are subject to reinfection later during the same breeding season or during subsequent seasons. The disease may be transmitted on farms utilizing artificial insemination by contaminated instruments and equipment. A stallion used for natural service must be sexually rested until the lesions have healed. Stallions with active disease used for artificial insemination may continue to breed if they do not hemorrhage during semen collection. An open-end artificial vagina can be used to collect the semen to prevent contact between the ejaculate and contaminated surfaces, thus reducing the risk of virus transmission to the mares being inseminated. Stallions can be asymptomatic carriers for a long period of time, and occasionally the virus can be isolated from external genital culture sites.

Trypanosoma equiperdum is the etiologic agent for dourine. Although not a problem in the U.S. breeding population, it should always be considered with the risks associated with shipping horses and semen across international borders. Clinical signs include fever, purulent exudate from the urethra of affected stallions, and chronic weight loss. Definitive diagnosis is based upon serologic testing and culture of the organism. Infected stallions do not respond to treatment and are often euthanized.

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CHAPTER 4

Surgical Correction of Abnormalities Affecting the Reproductive Organs of Stallions

JIM SCHUMACHER and DICKSON D. VARNER

INGUINAL HERNIATION AND RUPTURE

Inguinal herniation in the stallion occurs when intestine, usually the ileum or distal portion of the jejunum, enters the vaginal sac or cavity (i.e., the inguinal canal) through the vaginal ring. If intestine extends into the scrotum, this herniation is sometimes referred to as scrotal rather than inguinal,[1] but the terms are used interchangeably. Inguinal hernias of stallions are sometimes improperly referred to as "indirect hernias," a term used to describe a similar condition in human beings.

Ruptured inguinal (scrotal) herniation occurs when herniated intestine protrudes through a rent in the vaginal sac into the subcutaneous tissue of the scrotum.[2] Inguinal rupture is protrusion of viscera into the subcutaneous tissue of the inguinal canal or scrotum through a rent in the peritoneum and musculature adjacent to the vaginal ring.[1] Inguinal ruptures in horses are sometimes inappropriately termed "direct hernias," which is terminology borrowed from a somewhat similar condition in human beings. Direct hernias in human beings are caused by weakening of the inguinal musculature and are lined by peritoneum, whereas inguinal ruptures of horses are not lined by peritoneum. Direct herniation predominates in human beings, whereas inguinal herniation (i.e., indirect herniation) predominates in stallions.

Most inguinal hernias of foals are congenital and hereditary.[3] They occur when a vaginal ring is so large that it permits viscera to enter the vaginal sac. Congenital inguinal hernias in foals may occur unilaterally (usually on the left side) or bilaterally and may occur more frequently in Standardbreds, Tennessee Walking horses, and American saddlebred horses.[4,5]

Ruptured inguinal hernias occur most commonly in foals and may be caused by high abdominal pressure generated during parturition.[2,6] Inguinal hernias in adult stallions are generally acquired, but the underlying cause is usually a congenitally enlarged vaginal ring. The left vaginal ring is the ring most commonly involved.[5] Herniation has been reported to occur during breeding or

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Ruptured inguinal hernias occur most commonly in foals and may be caused by high abdominal pressure generated during parturition.[2,6] Inguinal hernias in adult stallions are generally acquired, but the underlying cause is usually a congenitally enlarged vaginal ring. The left vaginal ring is the ring most commonly involved.[5] Herniation has been reported to occur during breeding or

exercise,[7] but it has also been identified in stallions being transported and in stallions confined to a stall. The incidence of acquired inguinal herniation is higher in Standardbreds, American saddlebreds, and Tennessee Walking horses than in the general equine population,[4,7] and the higher incidence in these breeds may be attributable to herniation of viscera through a congenitally enlarged vaginal ring. Inguinal ruptures in horses are rare and usually occur after a traumatic event.[1]

Inguinal hernias, ruptured inguinal hernias, and inguinal ruptures usually cause a noticeable increase in the size of the scrotum. Palpation of the scrotum of an affected horse may elicit a sensation of crepitus, and peristalsis of entrapped intestine may cause movement of scrotal skin. The contents of congenital inguinal hernias in foals, because of the relatively large size of the vaginal ring, are rarely strangulated and reduce easily. Rupture of a congenital inguinal hernia should be suspected if the viscera cannot be reduced, if the scrotum is cold and edematous, or if the hernia is accompanied by signs of colic.[2,6]

Acquired inguinal herniation is usually first recognized when the affected stallion begins to show signs of severe colic caused by strangulation of incarcerated intestine by the vaginal ring. Acquired inguinal hernias are usually accompanied by scrotal and testicular edema, because the vasculature of the spermatic cord becomes obstructed. Examination of the stallion's vaginal rings by palpation per rectum reveals that intestine has entered a vaginal cavity. Omentum may also enter the vaginal cavity independently or with intestine, but incarceration of omentum alone is unlikely to be accompanied by signs of colic.

Foals with a congenital inguinal hernia should be monitored regularly by palpation for signs of strangulation. The hernia often resolves spontaneously by the time the foal is 3 to 6 months old, but the mechanism by which this occurs is not well understood.[1,8,9] Application of a truss may hasten resolution (Fig. 4-1). The truss is applied with the foal in dorsal recumbency after the hernia has been reduced manually. Care should be taken to avoid interfering with urination by compressing the penis with the truss. The truss is usually changed at 3- to 5-day intervals. The hernia is often corrected within 1 to 2 weeks following application of the truss.

Congenital inguinal hernias of foals usually resolve spontaneously, but surgical correction becomes necessary if the hernia is so large that it is unlikely to resolve spontaneously or if the contents of the hernia become strangulated. To surgically correct an inguinal hernia, the foal is anesthetized and positioned in dorsal recumbency, and the inguinal region is prepared for surgery. An incision is created over the superficial inguinal ring of the affected side, the vaginal sac and its contents are exposed by blunt dissection, and the scrotal ligament, which attaches the vaginal sac to the scrotum, is severed. The contents of the hernia are replaced through the vaginal ring into the abdomen, and the spermatic cord is ligated close to the superficial inguinal ring and severed distal to the ligature. The superficial inguinal ring can be sutured for added security, and the incised subcutaneous tissue and skin are sutured or left open to heal by second intention.

Congenital inguinal hernias of foals can also be corrected laparoscopically with the foal anesthetized and positioned in dorsal recumbency.[10,11] After the contents of the hernia are reduced, the testis of the affected side is either removed laparoscopically or left in situ, and the vaginal ring is closed with a laparoscopic stapling device. Laparoscopic correction of congenital inguinal herniation is relatively quick and allows the foal to return quickly to normal activity.

Stallions with an acquired inguinal hernia, a ruptured inguinal hernia, or an inguinal rupture demand immediate attention, because the intestinal contents of the hernia nearly always rapidly strangulate. Reduction by external manual manipulation or rectal traction on inguinally incarcerated intestine is described,[12] but is difficult and rarely successful. Surgical reduction of an acquired inguinal hernia is necessary if the viability of the affected testis or incarcerated intestine is uncertain.

To surgically reduce inguinally incarcerated intestine, the stallion should be anesthetized, positioned in dorsal recumbency, and prepared for inguinal exploration and celiotomy at the ventral midline. A cutaneous incision is

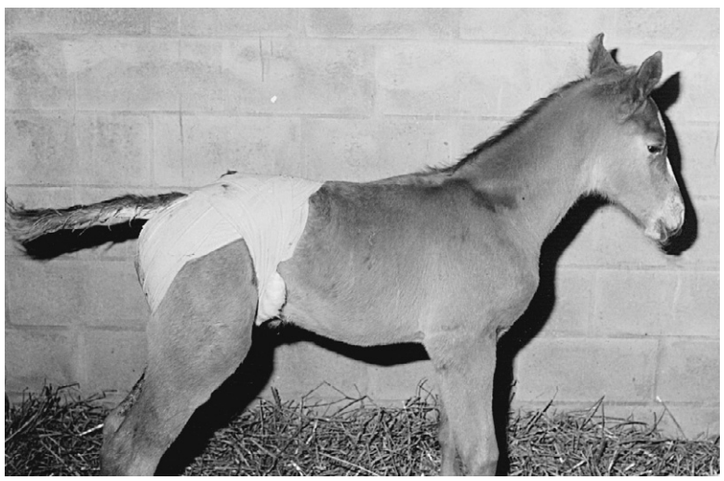

Fig. 4-1 "Diaper" applied to a foal to correct bilateral inguinal hernias. (From Varner DD, Schumacher J, Blanchard TL, Johnson L: *Diseases and Management of Breeding Stallions.* St. Louis, 1991, Mosby.)

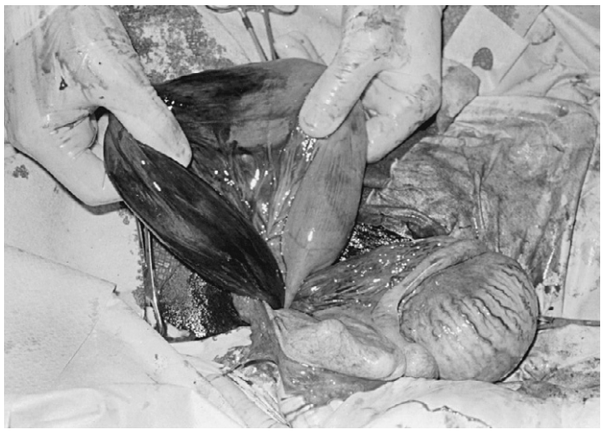

Fig. 4-2 Surgical correction of a unilateral scrotal hernia in a stallion. Note the discoloration of the entrapped intestine. (Courtesy of Dr. T.S. Ford; From Varner DD, Schumacher J, Blanchard TL, Johnson L: *Diseases and Management of Breeding Stallions.* St. Louis, 1991, Mosby.)

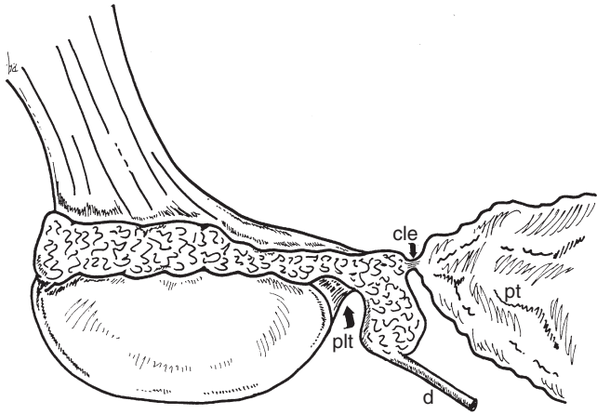

Fig. 4-3 Anatomy of the gubernaculum. *d,* Ductus deferens; *plt,* proper ligament of the testis; *cle,* caudal ligament of the epididymis; *pt,* parietal tunic.

created over the superficial inguinal ring or scrotum, the vaginal sac and its contents are isolated using blunt dissection, and the vaginal sac is incised to expose the testis, its spermatic cord, and the incarcerated intestine. Incarcerated intestine is most easily reduced by placing traction on it through a small ventral midline celiotomy. Often, the vaginal ring is so constricting that the intestine cannot be returned to the abdominal cavity without first enlarging the ring. The ring can be enlarged by cutting it with a curved bistoury or scissors.

Devitalized intestine (Fig. 4-2) can be resected and anastomosed at the inguinal incision, but resection and anastomosis are usually more easily accomplished after the intestine is returned to the abdominal cavity and exteriorized through a celiotomy at the ventral midline. Attempting to save the testis is impractical unless it and its vasculature appear to be undamaged. To remove the testis, the scrotal ligament is severed. The spermatic cord, including the parietal tunic, is ligated with heavy absorbable suture and severed distal to the ligature. The superficial inguinal ring is closed with a continuous or interrupted pattern using heavy absorbable suture. Inguinal fascia and skin can be sutured or left unsutured to heal by second intention. After removal of the affected testis, compensatory hypertrophy of the remaining testis may occur.[13]

TORSION OF THE SPERMATIC CORD

Torsion of the spermatic cord occurs when a portion of the spermatic cord rotates around its vertical axis.[14] Torsion of the spermatic cord is sometimes incorrectly referred to as testicular torsion. Torsions of 180 degrees or less seem to cause no discomfort to stallions and are often considered to be an incidental finding,[15] but torsion of a spermatic cord of 180 degrees may cause retrograde blood flow, indicating that torsion of this degree may have an injurious effect on testicular function.[16] Torsions of 360 degrees or more cause acute venous and arterial occlusion of testicular blood supply, and if the rotation is not reduced quickly, the testis and spermatic cord distal to the torsion become gangrenous.[14,15,17,18]

In human beings, torsion of the spermatic cord nearly always occurs intravaginally (within the vaginal tunic) rather than extravaginally (outside the vaginal tunic),[14] and likewise, torsion of the spermatic cord of the stallion apparently occurs primarily intravaginally.[15,17,18] Torsion may be caused by an abnormally long caudal ligament of the epididymis (ligament of the tail of the epididymis)[15] or by an abnormally long proper ligament of the testis (Fig. 4-3).[18] The gubernacular attachments of the contralateral testis may also be abnormally long, making that testis prone to torsion.[18] Torsion of the spermatic cord of horses is infrequently reported,[18,19] but torsion of 180 degrees is detected occasionally during examination of the external genitalia of the stallion. The spermatic cord of abdominally located testes may be more prone to torsion and may lead to gangrenous changes in the affected testis.[19]

Signs of torsion of the spermatic cord of stallions include scrotal swelling and signs of colic.[15,17,18] Other diseases, such as inguinal herniation, orchitis, and epididymitis, produce similar signs, but these diseases can usually be excluded by palpation of the scrotal contents, examination of the vaginal rings by palpation per rectum, and ultrasonography. Normally, the head of the epididymis lies on the cranial pole of the testis, the tail lies at the caudal pole, and the body attaches to the dorsolateral border of the testis. With torsion of 180 degrees, the tail of the epididymis lies cranial, but with torsion of 360 degrees, the testis and epididymis may appear to be correctly aligned. The affected testis and cord are enlarged and firm, and the testis may appear to be surrounded by fluid.[17,18] With torsion of 360 degrees or more, the scrotum and its contents may be so swollen that palpation of the epididymis is not possible. Ultrasonographic examination of the affected testis and cord may reveal increased fluid density throughout the affected side of the scrotum.[18] Torsion of the spermatic cord of an abdominal cryptorchid testis may cause the affected horse to show

mild signs of colic,[20] but affected horses may display no signs of colic.[21]

A 360-degree torsion of the spermatic cord typically necessitates removal of the affected testis. If the testis is salvageable, orchiopexy using nonabsorbable suture can be performed to permanently fix the testis in its proper position.[18] One suture is placed at the cranial aspect of testis and one at its caudal aspect. The suture is passed through the adjacent dartos tissue, vaginal tunic, and tunica albuginea. The surgeon should consider performing orchiopexy on the contralateral testis, because it may also be prone to torsion. Transient torsion of the spermatic cord of human beings may result in formation of antisperm antibodies or release of other factors that may affect fertility,[22] but the effect of transient torsion of the spermatic cord of stallions is not known.

HYDROCELE

A hydrocele is a pathologic accumulation of serous fluid between the visceral and parietal layers of the vaginal tunic.[23–26] Because of the insulating effects of the fluid, temperature-induced dysfunction of spermatogenesis is generally thought to occur, although one study did not reveal a substantial effect of hydrocele on semen quality. The vaginal tunic is secretory, and fluid produced by the vaginal tunic is resorbed through the veins and lymphatic vessels of the spermatic cord.[27] Hydrocele results when production of fluid by the vaginal tunic is increased or resorption is decreased. Hydroceles may accompany testicular neoplasia or scrotal trauma or they may be idiopathic. Some forms of idiopathic hydroceles may occur during hot weather, sometime in association with restricted exercise.[26] Because the vaginal cavity communicates with the peritoneal cavity, a hydrocele may form as an extension of ascites.[24] Migration of parasites into the vaginal cavity and associated structures has been implicated as a cause of hydrocele.[23]

A hydrocele appears as a painless, fluid-filled scrotal enlargement.[23–25,27] It may occur bilaterally or unilaterally and may develop acutely or insidiously. If the hydrocele is chronic, the testis within the affected tunic may become smaller than normal due to atrophy. A hydrocele can usually be differentiated from other diseases that cause scrotal enlargement by palpating the scrotal contents, by examining the vaginal rings per rectum, and by using ultrasonography. Ultrasonographic examination of a hydrocele reveals anechoic or hypoechoic fluid surrounding the testis (Fig. 4-4). Diagnosis is verified by aseptic aspiration of a serous, amber fluid from the vaginal cavity.

Exercise may cause a temporary decrease in the size of a hydrocele, and occasionally, a hydrocele may resolve spontaneously. Aspiration of fluid from a hydrocele usually gives only transient relief because the fluid soon re-forms. Treatment of a stallion with a hydrocele should be aimed at removing the inciting cause, but because the cause can rarely be identified, the usual treatment of a stallion with persistent, unilateral hydrocele is removal of the affected testis and vaginal tunic to avoid disruption of spermatogenesis of the contralateral testis that may be induced by increased scrotal temperature. Prognosis for fertility can be guarded if bilateral hydroceles persist, although the condition has been reported to have no ill effect on fertility in some cases.[26]

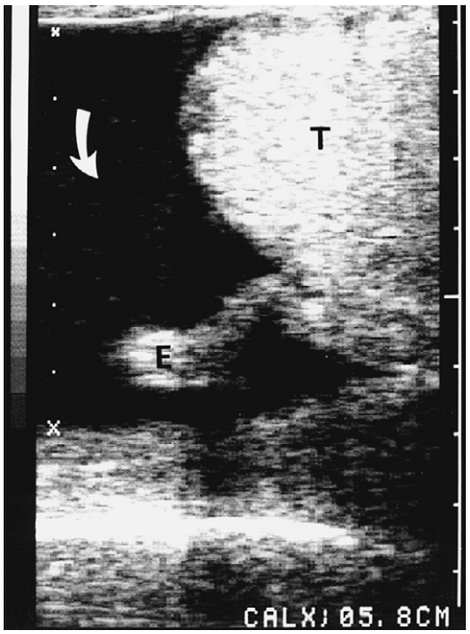

Fig. 4-4 Ultrasonographic image of the testis *(T),* cauda epididymis *(E),* and vaginal space *(arrow)* in a stallion with a pronounced hydrocele. (From Varner DD, Schumacher J, Blanchard TL, Johnson L: *Diseases and Management of Breeding Stallions.* St. Louis, 1991, Mosby.)

Hydroceles of men were successfully treated by sclerotherapy using either 2.5, 5, or 10% solutions of tetracycline injected into the vaginal cavity.[28] Injection of the sclerosant often caused severe pain, even if local anesthetic solution was injected into the vaginal cavity prior to treatment. Sclerotherapy, as a treatment for stallions with hydrocele, has not been reported.

HEMATOCELE

A hematocele resembles a hydrocele but is a collection of hemorrhagic, rather than serous, fluid within the vaginal cavity (Fig. 4-5).[25,29] Hematoceles are usually caused by trauma to the scrotum and its contents, but because the peritoneal and vaginal cavities of the horse communicate, they can occur as an extension of hemoperitoneum.[30]

A hematocele caused by acute trauma to the scrotal contents is usually accompanied by pain. Ultrasonographic examination of the contents of the scrotum may help differentiate hematocele from hydrocele and other causes of scrotal enlargement. Some causes of hematocele, such as rupture of the tunica albuginea of the testis, may be detected by ultrasonographic examination. Diagnosis is confirmed by aseptic aspiration of sanguineous fluid from the vaginal cavity. Scrotal swelling associated with hematocele can be quite pronounced.

A small hematocele may cause no problem with fertility and may dissipate without treatment, but a large hematocele may insulate the testes, causing interference

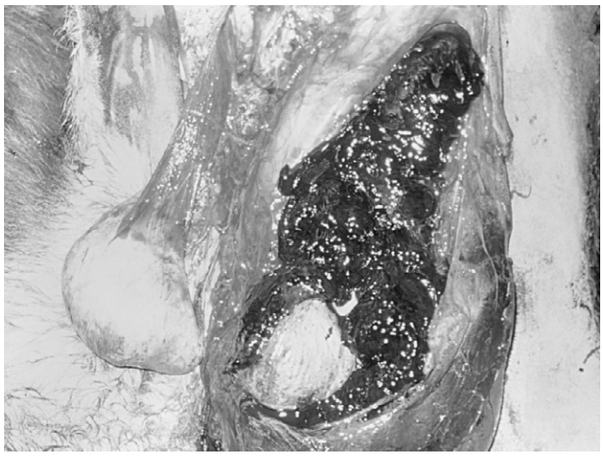

Fig. 4-5 Massive bleeding into the vaginal space of a jack emanating from a ruptured tunica albuginea and testicular hemorrhage. (From Varner DD, Schumacher J, Blanchard TL, Johnson L: *Diseases and Management of Breeding Stallions.* St. Louis, 1991, Mosby.)

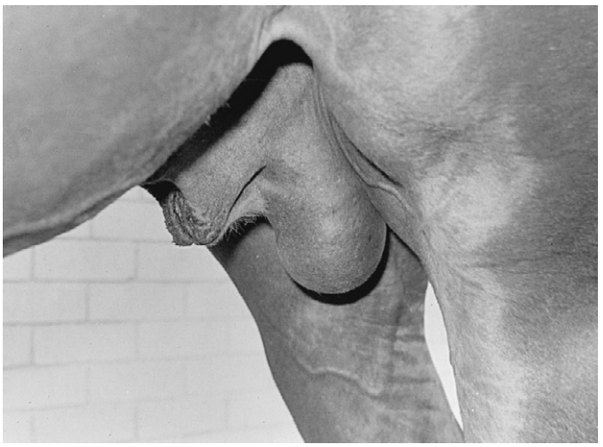

Fig. 4-6 Varicocele of the left pampiniform plexus in a stallion, resulting in abnormal thickening of the scrotal neck on the affected side. (From Varner DD, Schumacher J, Blanchard TL, Johnson L: *Diseases and Management of Breeding Stallions.* St. Louis, 1991, Mosby.)

with spermatogenesis. A large hematocele should be evacuated from the vaginal cavity, and the testis and epididymis should be carefully inspected to identify the source of hemorrhage. The tunica albuginea, if torn, should be sutured. Orchidectomy is indicated if the testis or epididymis is badly damaged. The effect of testicular trauma on formation of antisperm antibodies and secondary subfertility in stallions is not known. Removal of the affected testis may be indicated to minimize the likelihood of such a complication and to prevent depression of spermatogenesis of the contralateral testis from increased temperature caused by inflammation of the scrotal contents.

VARICOCELE

A varicocele is a dilatation and tortuosity of the veins of the pampiniform plexus and the cremasteric veins (Fig. 4-6).[24,25] Varicoceles of human beings and rams have been associated with testicular atrophy and decreased seminal quality, possibly caused by interference with normal exchange of heat from the testicular artery to the pampiniform plexus.[24] Varicoceles of stallions are uncommon, and their effect on fertility is not documented. More than 50% of men with varicoceles have normal seminal quality.[31] One of the authors (DV) has observed a varicocele in some stallions with normal seminal quality.

Most varicoceles are idiopathic, but a defect in the valves of the spermatic vein where it empties into the vena cava or renal vein, or a deficiency of elastic and fibrous tissue in the fascia that surrounds the spermatic vein has been postulated to cause varicoele.[24] Varicoceles of stallions are usually unilateral and, when palpated, produce no signs of pain. The affected spermatic cord appears enlarged and may have the texture of "a bag of worms."

Definitive treatment of stallions affected with varicocele is removal of the affected cord and testis, but therapy is unnecessary if seminal quality is unaffected. The venous loops of the pampiniform plexus have been ligated as treatment of human beings with varicocele.[32] A hydrocele has formed in a small percentage of human beings following ligation of the spermatic vein.

TESTICULAR NEOPLASIA

Testicular neoplasms of the horse are encountered infrequently, probably because most horses are castrated at an early age. Only primary testicular neoplasms (i.e., those that originate within the testis) have been reported, and these can be divided into germinal and nongerminal types.[33] Germinal neoplasms arise from the germ cells of the seminiferous epithelium and are the most common type of germinal neoplasm.[24]

Germinal testicular neoplasms reported to occur in the horse include the seminoma, teratoma, teratocarcinoma, and embryonal carcinoma. The seminoma is probably the most commonly reported testicular neoplasm of the horse. Nongerminal testicular neoplasms arise from testicular stromal cells and include the Leydig cell tumor and Sertoli cell tumor.[33] Nongerminal testicular neoplasms of the horse are uncommonly reported. Cryptorchid testes of human beings and dogs appear to be predisposed to neoplasia,[33,34] but for horses, a relationship between cryptorchidism and formation of testicular neoplasia has not been definitively established.

When examining a horse with a suspected testicular neoplasm, the clinician should use the contralateral testis for comparison, remembering, however, that bilateral testicular neoplasia can occur. The normal testis is smooth and compliant, and a neoplastic testis is often enlarged, with either a soft or hardened texture of the tumorous tissue. The neoplastic testis is often heavier than its normal counterpart. Small, neoplastic lesions located

deep within the parenchyma may not be palpable. A neoplastic testis is usually painless when compressed and usually remains freely movable within the scrotum. Scrotal enlargement caused by neoplasia must be differentiated from other causes of scrotal enlargement, such as torsion of the spermatic cord, orchitis, hydrocele, hematocele, and inguinal herniation or rupture. Careful external palpation of the scrotal contents and palpation of the vaginal rings and viscera per rectum can be used to differentiate these conditions. Painless, scrotal enlargement that develops insidiously is more likely to be caused by testicular neoplasia than by inflammation or ischemia.

Ultrasonographic examination of the contents of the scrotum may be helpful in determining if a testis is neoplastic. Normal testicular parenchyma is homogeneously echogenic, but a neoplastic testis usually contains areas of decreased echogenicity. Affected testes may contain single or multiple tumors. Testicular neoplasia can be confirmed by cytologic examination of a needle aspirate or by histologic examination of a needle or incisional biopsy of the testis. Incisional biopsy has been associated with complications, but needle biopsy is unlikely to have injurious effects on the testis.[35–38] Creation of antisperm antibodies and reduction in quality of semen reported to occur in other species after needle biopsy have not been found to occur in horses. Biopsy of neoplastic testes of human beings has been associated with a high incidence of neoplastic invasion of extratesticular tissue,[39] and so, if testicular neoplasia is strongly suspected, the affected testis should be excised.

Before a neoplastic testis is removed, the iliac lymph nodes should be palpated per rectum to aid determination of whether the neoplasm has metastasized (Fig. 4-7). The neoplastic testis should be removed using the closed technique, and the spermatic cord should be ligated and severed as far proximally as possible. The spermatic cord should be examined grossly and histologically for evidence of metastasis. The scrotal incision should be sutured to reduce postoperative inflammation, which may interfere with thermal regulation of the remaining testis, but good hemostasis is required before the scrotal incision is sutured.

PENILE AND PREPUTIAL INJURIES

Penile and preputial injuries include lacerations and hematomas and are usually caused by kicks, especially to the erect penis; mounting of stationary objects; attempting to breed a mare across a barrier; severe bending of the penile shaft during coitus or semen collection; and improperly fitted or maintained stallion rings.[40] Deep lacerations that extend into a corporeal body may result in hemospermia or impotence,[41] and those that extend into the urethra may result in severe necrosis of tissue from escape of urine.

Even superficial lacerations can result in severe penile damage if the horse is left untreated. An unattended laceration to the penile epithelium can result in cellulitis and preputial edema, which in turn, can lead to protrusion of the penis and internal preputial lamina from the preputial cavity. Protrusion of the penis and prepuce may lead to penile paralysis from damage to the pudendal nerves and to further damage to the exposed penile and preputial epithelium.

Fresh lacerations to penile and preputial epithelium should be sutured with soft, absorbable or nonabsorbable suture. An infected or heavily contaminated laceration should be left open to heal by second intention or closed at a later date when it shows no signs of inflammation. If the wound is left open, it should be dressed frequently with a nonirritating antimicrobial ointment. If the laceration is accompanied by severe preputial edema, the penis and prepuce should be retained within the preputial cavity with a retainer bottle, nylon netting, or hosiery suspended at the preputial orifice with a crupper and surcingle made of rubber tubing (Figs. 4-8 and 4-9).

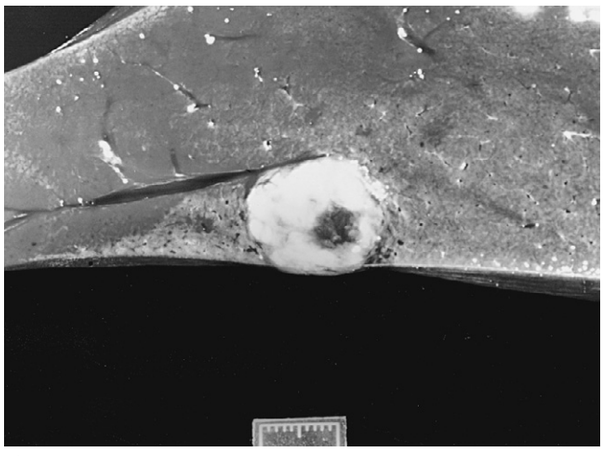

Fig. 4-7 Metastatic seminoma of liver. (Courtesy of B. Smith, DVM.)

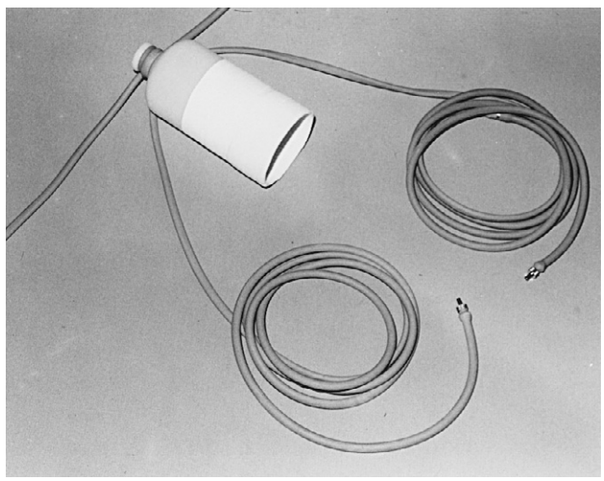

Fig. 4-8 Device fabricated for retaining the penis within the preputial cavity of stallions with penile prolapse. (From Varner DD, Schumacher J, Blanchard TL, Johnson L: *Diseases and Management of Breeding Stallions.* St. Louis, 1991, Mosby.)

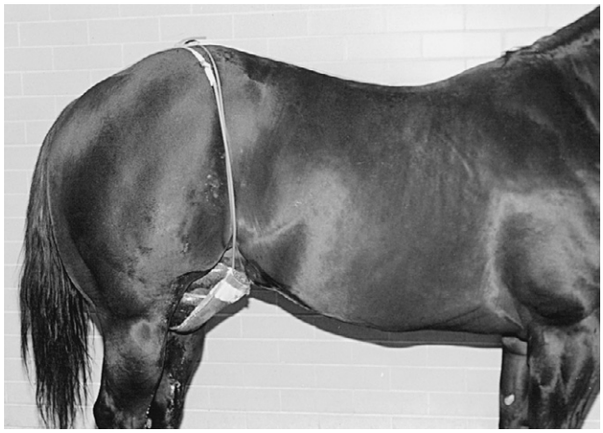

Fig. 4-9 Nylon mesh, with attached tubing, fitted over the preputial orifice of a stallion to aid retention of the device shown in Figure 4-8 within the preputial cavity. (From Varner DD, Schumacher J, Blanchard TL, Johnson L: *Diseases and Management of Breeding Stallions.* St. Louis, 1991, Mosby.)

Fig. 4-10 A bandage is used to compress the penis against the ventral body wall.

The penis can be retained within the preputial cavity for several days with towel clamps or sutures placed across the preputial orifice or preputial ring, but sutures and towel clamps may exacerbate the preputial trauma. If the penis cannot be retained in the preputial cavity, it can be compressed against the ventral aspect of the belly wall with a bandage (Fig. 4-10).

Penile hematomas that continue to expand should be explored to identify a rent in the tunica albuginea. A laceration to the tunica albuginea should be identified and sutured. A laceration to the urethra should be sutured, and if the laceration is transverse, a large-bore catheter should be maintained in the lumen of the urethra to prevent formation of a stenosing cicatrix. A stallion affected with preputial and penile trauma should be isolated from mares until the wound has healed.

PARAPHIMOSIS OR PENILE PROTRUSION

Paraphimosis, or the inability of the horse to retract its penis into the preputial cavity, is usually caused by preputial edema that occurs secondary to trauma, such as preputial laceration or preputial hematoma, or from preputial edema that accompanies systemic disease such as dourine or purpura hemorrhagica.[40,42] Penile protrusion can be caused by damage to innervation of the penis, which may accompany some neurologic diseases, such as equine herpesvirus 1.[40,43] Penile protrusion has been associated with severe debilitation[44] and with administration of phenothiazine-derivative tranquilizers.[45,46] A phenothiazine-derivative tranquilizer can, though rarely, cause penile paralysis or priapism, especially in stallions, and for this reason, phenothiazine-derivative tranquilizers probably should not be administered to stallions.

Inability of a stallion to maintain its penis and prepuce with the preputial cavity, regardless of the cause, impairs venous and lymphatic drainage of the penis and prepuce, which leads to edema of the internal preputial lamina. As the internal preputial lamina swells, the preputial ring may become constricting, causing the penis distal to the ring to swell further. The exposed penile and preputial epithelium, if not protected, becomes excoriated and infected. Eventually, the internal preputial lamina becomes fibrotic, and the prepuce loses its normal telescoping action. In addition, the pendulous weight of the protruded penis and prepuce may damage the internal pudendal nerves, causing penile paralysis.[45]

The protruded penis should be replaced into the preputial cavity as soon as possible to protect it and the internal preputial lamina from further injury, but if the penis and prepuce are so edematous that they cannot be replaced into the preputial cavity, they should be compressed against the ventral aspect of the body wall with a bandage. The penis and prepuce should be kept lubricated with an emollient, antimicrobial dressing. The horse should receive a nonsteroidal, anti-inflammatory drug and should be exercised daily. If the preputial ring restricts penile retraction or impairs venous and lymphatic flow, it should be incised longitudinally.[43] This incision is allowed to heal by second intention.

If paraphimosis has caused permanent penile paralysis, the stallion is unlikely to be able to achieve erection. If a stallion with penile paralysis can ejaculate into an artificial vagina, and if the stallion's breed registry permits artificial insemination, the stallion's breeding life can be extended through artificial insemination of mares.[40,47] The stallion can be salvaged for uses other than breeding through amputation of the penis or permanent retraction of the paralyzed penis into the prepuce.

PHIMOSIS

Phimosis, or the inability of the horse to completely protrude its penis from the prepuce, occurs naturally in foals, because the internal preputial lamina is fused to the free part of the penis for about the first month after birth.[48] Excluding this normal physiologic condition, phimosis is usually the result of constriction of the external preputial orifice or the preputial ring caused by trauma or

neoplasia. When the horse is unable to protrude its penis, it urinates within the preputial cavity, causing excoriation of the preputial epithelium, which leads to further inflammation and scar formation, thereby compounding the problem.

A constricting external preputial orifice can be enlarged by removing a triangular segment of external lamina, whose base is the preputial orifice.[49] The cut edge of the external lamina is sutured to the cut edge of the internal preputial lamina. A constricting preputial ring can be opened by incising the internal preputial fold. After the penis is protruded, the constricting cicatrix can be removed by segmental posthectomy (i.e., reefing). See the next section for a description of the reefing operation.

NEOPLASIA OF THE PENIS AND PREPUCE

Any cutaneous neoplasm can affect the integument of the external genitalia, but the most common neoplasm of the penis and prepuce is the squamous cell carcinoma.[30,43,50] Lesions of squamous cell carcinoma are usually multiple and often involve the glans penis and the internal lamina of the prepuce.[24,51] Geldings develop squamous cell carcinoma of the genitalia more frequently than do stallions, and horses with nonpigmented genitalia, such as Appaloosas and American Paint horses, are most frequently affected.[43,51,52] Malignancy of squamous cell carcinomas of the penis and prepuce of the horse is usually low grade, and lesions tend to remain localized, but lymphatic spread may occur, first to the inguinal lymph nodes, if treatment of lesions is neglected.[24,51] Penile or preputial squamous cell carcinoma can metastasize to internal organs without causing detectable enlargement of the inguinal lymph nodes,[53] and slight metastatic enlargement of the inguinal lymph nodes may be difficult to distinguish from lymphadenopathy secondary to balanoposthitis.[54] Prognosis for survival is poor if squamous cell carcinoma has metastasized to the inguinal lymph nodes, and invasion of squamous cell carcinoma into a corporeal body is likely to result in hematogenous spread of the neoplasm to internal organs.[55] Before treatment of the horse for penile or preputial neoplasia is undertaken, the superficial inguinal lymph nodes should be examined to detect enlargement; the penile shaft should be palpated for evidence of invasion of a corporeal body; and the stallion's body condition should be observed for evidence of metastases.

Small preputial and penile neoplasms of stallions can be excised or destroyed using cryotherapy. Cryotherapy can be performed using liquid nitrogen administered as a spray or through a cryoprobe or with carbon dioxide administered through a cryoprobe (Fig. 4-11).[56,57] Lesions should be frozen to –20° C using two or three freeze-thaw cycles. A rapid freeze and slow thaw produce the most cellular damage. A thermocouple can be used to monitor the size and depth of the area affected by the cryogen.

Radiofrequency-induced hyperthermia has been used to treat horses affected with sarcoids and cattle and horses affected with ocular squamous cell carcinoma,[58,59] but its use for treatment of genital squamous cell carcinoma of horses has not been evaluated. Application of hyperthermia to a penile or preputial neoplasm may enhance the effect of chemotherapeutic agents.[59]

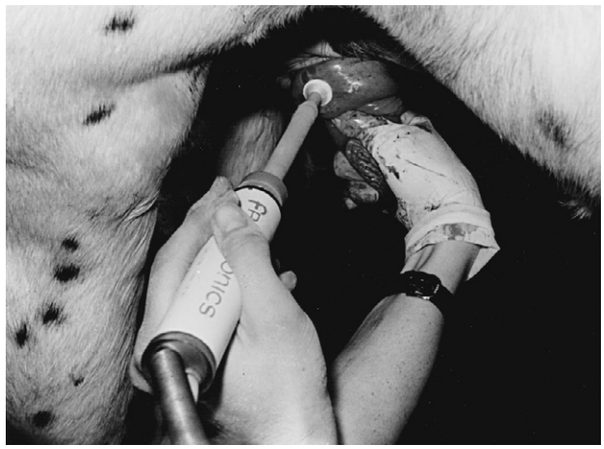

Fig. 4-11 Cryotherapy using a probe to treat a lesion of squamous cell carcinoma of the internal lamina of the prepuce.

Horses with precancerous and small lesions (<2–3 mm) of squamous cell carcinoma can be treated by application of 5% 5-fluorouracil at 14-day intervals.[60] Up to seven treatments may be necessary to bring about resolution of lesions. Horses with squamous carcinoma of the penis and prepuce have been treated successfully by intratumoral injection of cisplatin in sesame oil.[61,62] Affected horses are usually treated four times at 2-week intervals. Horses affected by metastasis of squamous cell carcinoma to internal organs can be treated by systemic administration of a chemotherapeutic agent, such as doxorubicin or piroxicam,[63] but little information is available about the efficacy of this treatment.

Stallions with extensive lesions of the internal lamina of the prepuce may require segmental posthectomy (i.e., reefing). To reef the internal lamina of the prepuce, the horse is anesthetized, positioned in dorsal or lateral recumbency, and prepared for surgery. The penis is extended by traction, and the urethra is catheterized. If desired, a tourniquet can be placed proximal to the surgical site. Parallel, circumferential, cutaneous incisions are made proximal and distal to the preputial lesion, and these incisions are connected by a longitudinal incision (Fig. 4-12). The diseased segment of prepuce between the circumferential incisions is removed from the penis using scissor dissection. All bleeding vessels are ligated with absorbable suture, and loose fascia is apposed with interrupted 0 or 2-0 absorbable sutures. Care must be taken to maintain the prepuce in proper alignment, and placing a suture in the fascia at four equidistant points around the circumference of the penis may aid in orientation. The integument is apposed with interrupted 0 or 2-0 absorbable or nonabsorbable sutures. The amount of internal lamina that can be surgically excised without disrupting normal copulatory function is not known.

To prevent disruption of sutures caused by penile erection, the stallion should be isolated from mares for at least 2 weeks, and application of a stallion-ring to the penis may be necessary. The stallion should be exercised daily

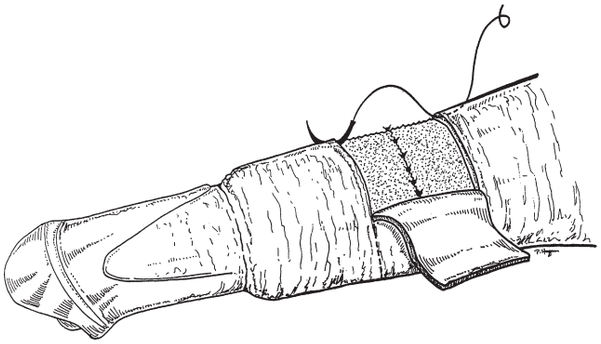

Fig. 4-12 Segmental posthectomy. A cuff of epithelium is removed from the internal lamina of the prepuce.

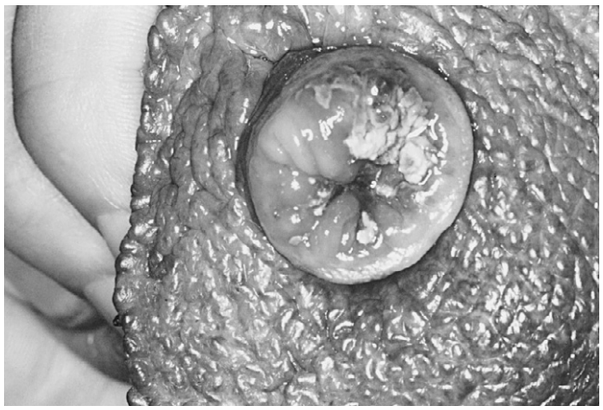

Fig. 4-13 Caseous masses associated with cutaneous habronemiasis at the mucocutaneous junction of the distal urethra. (From Varner DD, Schumacher J, Blanchard TL, Johnson L: *Diseases and Management of Breeding Stallions.* St. Louis, 1991, Mosby.)

to reduce postsurgical edema. Nonabsorbable sutures should be removed at 10 to 12 days.

Phallectomy of stallions may be indicated if neoplasia is extensive or has invaded the tunica albuginea, but phallectomy should only be considered as a salvage procedure. The horse should be castrated at least 2 weeks prior to phallectomy, if possible, and separated from other horses for 2 weeks after phallectomy to decrease the likelihood of sexually induced erection and disruption of sutures.

SUMMER SORES (CUTANEOUS HABRONEMIASIS)

Summer sores are pruritic, pyogranulomatous lesions caused by aberrant, cutaneous migration of the larvae of the equine stomach worm, *Habronema*.[64,65] Summer sores, or cutaneous habronemiasis, can be found anywhere on the integument, and the genitalia, especially the urethral process or preputial ring, are common sites of infestation (Fig. 4-13). Summer sores appear in the warm months of the year when flies, which are the nematode's intermediate host, are prevalent. Summer sores may disappear with the advent of cold weather. Horses that are prone to expose their penises are particularly vulnerable to cutaneous habronemiasis of the genitalia.

Migration and encystment of the larvae of *Habronema* invoke an inflammatory response from the host causing formation of exuberant granulation tissue that is characterized by the presence of small, yellow, caseous granules. Skin surrounding the granulation tissue may be depigmented.[65] Lesions of the preputial ring may interfere with the normal telescoping action of the prepuce, and lesions of the urethral process may involve the corpus spongiosum penis, causing hematuria or hemospermia.[66]

Summer sores may resemble lesions of pythiosis, carcinoma, the fibroblastic form of sarcoid, or exuberant granulation tissue caused by trauma. The presence of small caseous granules in the lesion usually enables the condition to be differentiated from other diseases with similar appearance. When the lesion is squeezed, larvae can occasionally be extruded onto a slide and identified microscopically.[64] A histologic characteristic of lesions is granulation tissue permeated with eosinophils, granules, and larvae. Affected horses often have a marked eosinophilia.

Ivermectin, administered systemically, or an organophosphate, administered systemically or topically, has been effective in resolving lesions by eliminating the migrating larvae. Corticosteroids or diethylcarbamazine, administered systemically, have been successful in resolving lesions by eliminating the horse's response to the larvae.

Lesions of habronemiasis of the internal lamina of the prepuce can be surgically excised. Small lesions can be removed by elliptical excision, but large or multiple lesions are often best removed by reefing. Lesions of the urethral process may necessitate amputation of the urethral process.[64,66] The urethral process can be amputated with the horse anesthetized or with the horse standing and sedated. A male urinary catheter is passed into the urethra, and if surgery is performed with the horse standing, the base of the urethral process is injected with local anesthetic. The urethral process is circumferentially excised proximal to the lesion, and the urethral mucosa is sutured to the epithelium of the stump of the urethral process to close the exposed corpus spongiosum penis (CSP) using 2-0 or 3-0 absorbable suture. Fibrous tissue at the suture site may tear during subsequent breeding, causing hemospermia.

PRIAPISM

Priapism, or persistent erection without sexual arousal, occurs when detumescence of the engorged corpus cavernosum penis (CCP) fails because of disturbances of arterial inflow or venous outflow (Fig. 4-14).[67] Priapism occurs in both stallions and geldings, but stallions are more frequently affected. The condition is economically devastating when a valuable breeding stallion is affected, because impotence is the usual outcome, and phallectomy may be required.

Priapism in human beings has many causes, but in horses, priapism is usually caused by administration of a phenothiazine-derivative tranquilizer.[68,69] Phenothiazine-

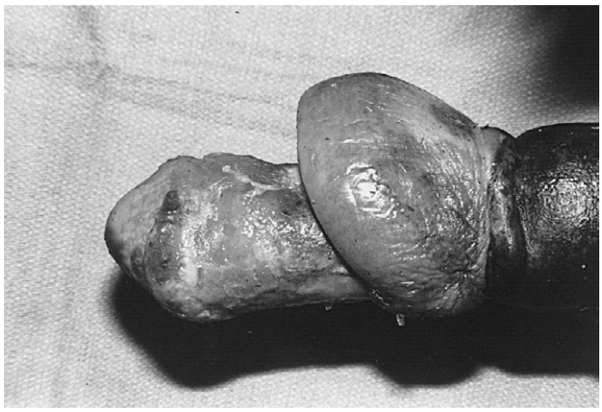

Fig. 4-14 Penis of a horse suffering from priapism. The corpus spongiosum is not involved in the erection. The preputial ring has become edematous.

derivative tranquilizers may cause priapism by blocking sympathetic impulses that initiate detumescence.[70] Other, less frequently reported causes of priapism in horses include tumors of the pelvic canal,[71] general anesthesia,[72] and nematodiasis of the spinal cord.[73]

The precise mechanism by which priapism occurs is not known, but basically, priapism of horses results from a disturbance of venous outflow to the CCP, causing the erect penis to fail to detumesce. The corpus spongiosum penis (CSP) remains uninvolved. When detumescence fails, blood in the CCP becomes stagnant, and partial pressure of CO_2 in the stagnant blood rises, causing erythrocytes to sickle.[74] The sickled erythrocytes obstruct venous outflow from the CCP, and the collecting veins eventually become irreversibly occluded.

Arterial supply to the CCP is still patent in the early stages of priapism, but if priapism persists, it too becomes irreversibly occluded.[74] Eventually, the trabeculae of the cavernosal tissue become fibrotic and lose their expansible capacity necessary for normal erection. In addition to damaging erectile tissue, prolonged erection in the horse may also result in penile paralysis by damaging the pudendal nerves, perhaps by compressing the nerves against the ischium (authors, personal observation). The eventual outcome of unresolved priapism in the horse is impotence caused by loss of both erectile function and sensitivity of the penis.

The primary clinical sign of priapism is prolonged protrusion of a turgid penis. Although the affected horse may not have a full erection, turgidity of the CCP can be detected when the penis is palpated. The penile and preputial epithelium becomes edematous soon after the onset of priapism[75] and, if not protected, becomes excoriated and infected. Dysuria may accompany the condition in some horses.[75] The CCP of a horse with chronically unresolved priapism feels fibrous and appears densely echogenic when examined ultrasonographically. A chronically affected horse may fail to respond to painful stimuli applied to the distal aspect of the penis.[71,76]

Horses with priapism have been treated empirically with diuretics, corticosteroids, general and regional anesthesia, penile and preputial massage, emollient dressings, and slings, but success in resolving priapism using these treatments is poor.[75] Although massage, dressings, and slings are ineffective in resolving priapism, they are important in preventing damage to the exposed organ.[75] Benztropine mesylate, a cholinergic blocker, has been used successfully to treat horses affected with priapism.[72,77] Affected horses of average size have been treated with 8 mg, administered by slow intravenous injection. Alpha-adrenergic agents, such as adrenalin and phenylephrine have been injected into the CCP of human beings to bring about detumescence,[78] and instillation of 10 mg of phenylephrine diluted in physiologic saline solution (10 ml) directly into the erect CCP of horses affected with priapism will usually bring about detumescence This response can be corrective for horses affected acutely by priapism but is usually a temporary response (2 to 4 hours) for horses affected chronically.

If medical therapy is unsuccessful in bringing about detumescence, the horse should be treated by irrigation of the CCP to evacuate sludged blood. Heparinized saline (10 units/ml physiologic saline solution) is injected through a large-bore needle inserted into the erect CCP proximal to the glans penis. Sludged blood and physiologic saline solution are evacuated 10 to 15 cm caudal to the scrotum through a small stab incision or through one or two large-bore needles inserted into the CCP. The CCP is irrigated until fresh hemorrhage appears in the efflux. The stab incision in the tunica albuginea of the CCP should be sutured after irrigation. If arterial blood fails to appear after irrigation, arteriolar supply to the CCP is probably permanently damaged, and impotence is likely. Failure of erection to subside following irrigation indicates that arteriolar inflow is patent and that venous outflow is still occluded.[79]

If erection recurs after irrigation of the CCP, establishing an alternative route of exit for the sludged blood may be necessary to bring about detumescence. The shunt should be created before the corporeal tissue and pudendal nerves are irreversibly damaged, but the time at which damage becomes irreversible has not been established for the horse. Irrigation of the CCP likely postpones the time at which the corporeal tissue becomes irreversibly damaged. The CCP can be anastomosed to the CSP to create a shunt for escape of venous blood trapped in the CCP (Fig. 4-15). The CSP may offer a convenient outlet for trapped venous blood, because its venous outflow differs from that of the CCP, and in contrast to the CCP, the CSP does not act as a closed system during erection. Impotence has occurred after creation of the shunt in human beings from failure to achieve or maintain pressure in the CCP necessary for intromission,[67,79] and this failure can result from damaged cavernosal tissue or from the shunt.[79] The shunt may close as normal venous outflow resumes, but whether closure is necessary for erection to be reestablished and maintained has not been determined.[80] A shunt between the CCP and CSP following trauma to the penis apparently causes impotence in the bull,[81,82] but studies of normal stallions that had a shunt created surgically between the CCP and CSP suggest that a shunt between the CCP and the CSP of

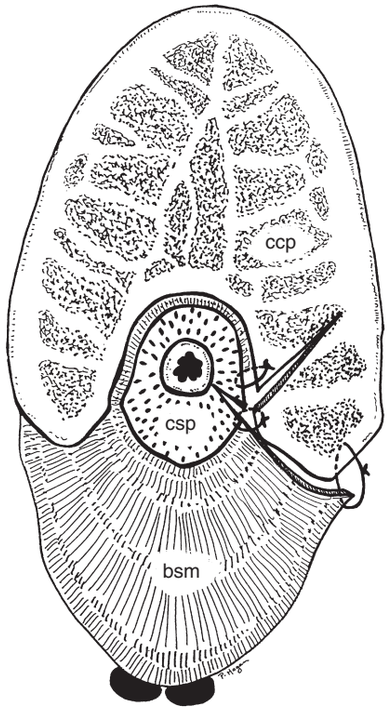

Fig. 4-15 Cross section of penis showing creation of a shunt between the corpus cavernosum penis *(ccp)* and the corpus spongiosum penis *(csp).* The cavernous tissue is exposed by elevation of the bulbospongiosus muscle *(bsm).*

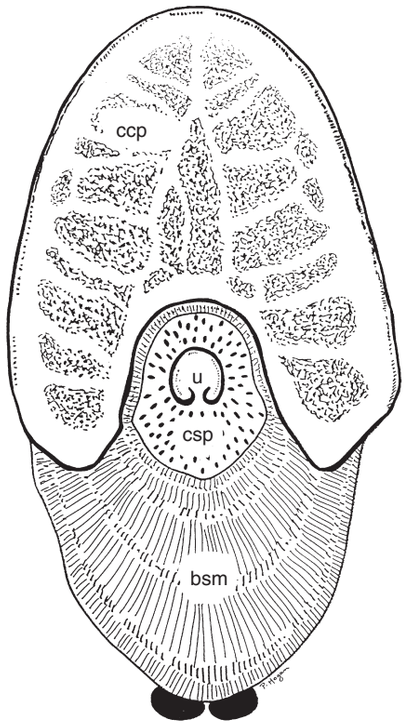

Fig. 4-16 Cross section of penis showing a defect in the urethra. *ccp,* Corpus cavernosum penis; *csp,* corpus spongiosum penis; *bsm,* bulbospongiosus muscle; *u,* urethra.

stallions does not interfere with subsequent erection and ejaculation.[83]

To create the shunt, the horse is anesthetized, positioned in dorsal recumbency, and prepared for aseptic surgery, and a male urinary catheter is inserted into the urethra. A 15-cm, longitudinal midline incision is created 5 cm caudal to the base of the scrotum to expose the shaft of the penis. The right or left edge of the bulbospongiosus muscle is elevated from the underlying tunica albuginea to expose a 5-cm long section of the CSP. A 3-cm, longitudinal incision is made through the tunica albuginea of the exposed portion of the CSP, taking care to avoid penetrating the catheterized urethra, and an identical, 3-cm, longitudinal incision is made through the tunica albuginea of the adjacent CCP. Hemorrhage from the incised CSP is copious, and so to preserve visibility, suction becomes necessary at this point in the procedure.

The inner edge of the incision through the tunica albuginea of the CSP is sutured to the inner edge of the incision through the tunica albuginea of the CCP with 2-0 absorbable suture using a continuous pattern. To complete the vascular shunt, the outer edge of the incision through the tunica albuginea of the CSP is sutured to the outer edge of the incision through the tunica albuginea of the CCP. The bulbospongiosus muscle is sutured to the tunica albuginea of the CCP with 2-0 absorbable suture using a continuous pattern, and the subcutaneous tissue and skin are apposed separately in a routine manner. The stallion should be isolated from mares for a month after surgery to reduce the probability of erection.

Some stallions with permanently damaged erectile tissue and impaired penile sensitivity may be trained to ejaculate into an artificial vagina, and some may be able to achieve intromission if intromission into the vagina of the mare can be assisted. To lower the ejaculatory threshold of stallions, an antidepressive drug, such as imipramine, can be administered before breeding.[84]

HEMOSPERMIA

Hemospermia, a cause of infertility or reduced fertility of stallions, has been attributed to bacterial and viral urethritis, improperly applied stallion rings, habronemiasis or carcinoma of the urethral process, and wounds to the glans penis or seminal vesiculitis.[43,85-88] Hemospermia can also be caused by a urethral rent, the cause of which is unknown.[89] The usual source of voluminous hemorrhage is the CSP (Fig. 4-16). Hemorrhage typically occurs near the end of ejaculation when contraction of the bulbospongiosus muscle causes pressure within the CSP to increase from around 17 mm Hg to 1000 mm Hg.[90]

Hemospermia may occur more commonly in frequently bred stallions.[86,91] Affected stallions are sometimes slow to ejaculate, and ejaculation sometimes appears to cause pain.[86] Hemospermia is usually diagnosed by gross or microscopic examination of semen that has been collected with an artificial vagina. The site of hemorrhage can often be determined by examining the

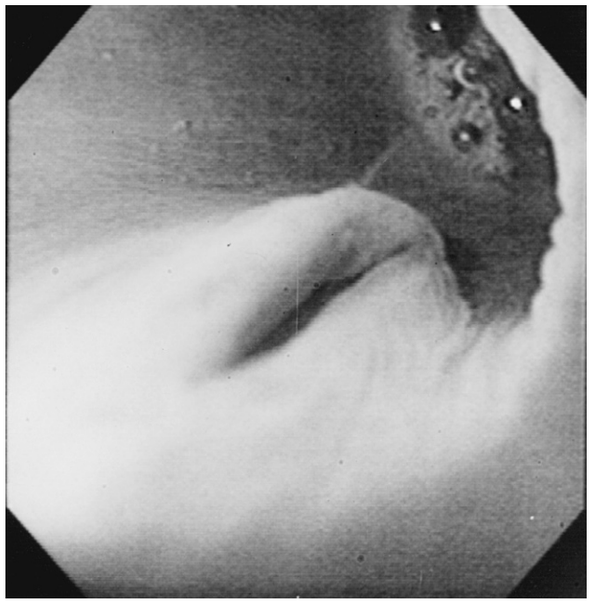

Fig. 4-17 Endoscopic view of a longitudinal defect at the convex surface of the urethra at the level of the ischial arch.

exterior of the penis or by examining the urethra with a sterilized, flexible endoscope that is at least 100 cm long. Endoscopic examination of the urethra of stallions affected with hemospermia often reveals a 5- to 15-mm longitudinal defect on the caudal surface of the urethra at the level of the ischial arch (Fig. 4-17). No gross signs of inflammation surround the defect.

Stallions affected with hemospermia have been treated medically by sexual abstinence and systemically administered formalin, methenamine, or antimicrobial drugs.[87] Enforcing sexual abstinence for a protracted time (exceeding 1 year) is often an unsuccessful treatment of stallions affected with hemospermia associated with urethral rents. Stallions affected with this form of hemospermia seem to be most effectively treated by temporary urethrotomy performed near the level of the ischial arch.[87,89,91] Urethrotomy is performed with the horse standing and sedated after administering epidural anesthesia. To facilitate identification of the urethra during dissection, a urethral catheter or the insertion tube of an endoscope is inserted into the urethra and advanced until it is proximal to the defect.

An 8- to 10-cm longitudinal incision, centered on the ischial arch, is created on the perineal raphe. The incision extends through skin, retractor penis and bulbospongiosus muscles, CSP, and urethral mucosa. Incising the urethral mucosa may be unnecessary, and opening the CSP without entering the urethra may reduce risk of complications associated with urethrotomy, such as a urethral fistula or stricture. The ischial wound is allowed to heal by second intention. Daily instillation of suppositories composed of an antimicrobial drug and a corticosteroid into the urethral lumen has been advocated[91] but is probably not necessary.

Stallions should receive sexual rest for at least 2 to 3 months after surgery. Horses may bleed at the urethrotomy for more than a week after surgery, especially at the end of urination when the bulbospongiosus muscle contracts. The ischial wound generally heals within 3 weeks.

Incising the CSP at the ischium decreases cavernosal pressure at the end of urination, and decreased pressure in the CSP may be responsible for the apparent success of temporary urethrotomy in eliminating hemospermia associated with urethral rents. When the bladder has emptied, the bulbospongiosus muscle contracts to expel urine that remains in the urethra, and these contractions increase pressure within the CSP.[92] Blood may be forced to exit the urethral rent at the end of each urination, because of increased pressure in the CSP, preventing the rent from healing. Incising the CSP converts this semiclosed vascular space into an open space, and during urination, blood flow is diverted from the urethral lesion to the urethrotomy, thus permitting the rent to heal.

Because the urethral defects are typically located at the caudal surface of the urethra near the ischial arch and are accessible through ischial urethrotomy, primary closure of the defect may be possible. Urethral endoscopy aids the surgeon in identifying the exact location of the defect. To confirm the location of the defect, a hypodermic needle can be inserted percutaneously into the lumen of the urethra at the level of the ischial arch during endoscopic examination.

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CHAPTER 5

Techniques for Artificial Insemination

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Although artificial insemination (AI) in horses can be done with semen collected at the farm for immediate use, maximization of this technology is achieved when mares are bred at considerable distances from where the stallion stands. To achieve this, semen either can be shipped in its liquid state after passive cooling and deposited in the mare several hours (12–36 hours) after collection or can be processed for freezing and maintained in liquid nitrogen until its use several weeks or years later. This chapter will discuss semen collection, handling and processing of cooled shipped and frozen semen, and the factors that affect the success of AI programs.

SEMEN COLLECTION

Breeding stallions that are going to be used for AI should have a semen evaluation performed prior to each breeding season. Because it is virtually impossible to improve the inherent quality of semen from a stallion, it is important for the mare owners as well as the stud farm to be aware of the stallion's semen quality. A veterinarian working for clients that decide to stand a stallion or stallions for AI has the responsibility of overseeing that the clients are properly set up for what they are offering. This preparation includes proper stimulus for the stallion such as, if necessary, the availability of a mare in heat at all times during the breeding season, availability of shipping containers, and a laboratory for the proper evaluation and processing of the semen. It is also important to inform the stallion owner that a proper semen evaluation should be performed to determine semen quality and microbiologic status of the semen including equine viral arteritis.

Stallions can be trained to achieve an erection by conditioning them to certain routines or by exposure to certain objects or animals associated with breeding. A stallion can be stimulated to achieve an erection by exposure to a mare in natural estrus, an ovariectomized mare that has been injected with estrogens, or a phantom or dummy mare where he is normally collected. In addition, stallions can be manually stimulated to drop their penis and then massaging the glans will elicit a full erection and pelvic thrusting movements. The stallion when collected with a properly prepared artificial vagina (AV) will follow a similar pattern of thrusting to that of natural mating.

To ejaculate into an AV it is not necessary for the stallion to jump a mare or a dummy. Collection on the ground can be achieved by teasing the stallion in cross ties, in an open area, or in a stall. Once the stallion has achieved a full erection, his penis is washed with clean water, dried and inserted into a properly prepared AV. The stallion in many instances will vocalize and move forward until the pelvic thrusts start at which time he might lift his front legs slightly or arch his back and have all four limbs on the ground until ejaculation. Most stallions with good libido can be easily trained to ejaculate on the ground.

It is the belief of many horse owners whose stallion(s) breed only by natural cover that a number of stallions cannot be collected artificially. However in the author's experience most stallions can be collected with an AV. Several models of AVs are available on the market, and although each has advantages and disadvantages, each operator should choose a model based on stallion suitability and ease of use. All AVs work under the same principle, which consists of a double rubber liner, usually latex, that will provide space to form a water jacket that at the adequate temperature and pressure will stimulate the stallion to ejaculate. Contrary to what would be expected, the temperature in the water jacket to stimulate a stallion to ejaculate is not body temperature but in general should be at least 42 to 43°C. In addition, older stallions often require temperatures of over 45°C to be properly stimulated. Furthermore, proper lubrication with a nonspermicidal water-soluble lubricant and the proper pressure are critical for adequate stallion stimulation. For stallions that have difficulty ejaculating into the AV, use of hot towels at the base of the penis to provide additional stimulation has been successful.

Although the author prefers the Missouri model AV due to its light weight and convenience of assembly and cleaning, there are other alternatives for semen collection. Condoms provide an alternative for obtaining a semen sample, particularly from stallions that are reluctant to ejaculate in an AV. Other methods of semen collection include the use of pharmacologic agents. These methods to induce ejaculation involve the use of pharmacologic regimens to either reduce the ejaculatory threshold in stallions or to induce extracopular ejaculation. Reduction of the ejaculatory threshold can be achieved with the use of diazepam 0.05 mg/kg IV or imipramine 500 to 1000 mg orally 15 to 30 minutes prior to breeding. Pharmacologic induction of ejaculation is achieved by slow IV injection of xylazine (0.66 mg/kg) with or without prior administration of 2.2 mg/kg of imipramine IV. In general, pharmacologic induction of

ejaculation occurs within a few minutes of the injection and the success rate is about 30 to 50%. It must be pointed out that this method does not always result in ejaculation from the same stallion. Therefore, it is difficult to program breedings if pharmacologic ejaculation is the only method available for semen collection.

Because many stallions breed a reduced number of mares and therefore ejaculate only a few times during the breeding season, it is important to identify stallions that are "sperm accumulators." These stallions in general have large testicles, and if sperm is not collected on a regular basis, they have the tendency to accumulate sperm in their reproductive tract. The accumulated sperm tend to have a lower motility, reduced longevity, and poorer morphology. Therefore, when such a stallion is offered for AI it is important to collect him several times prior to breeding mares, shipping, or processing for freezing. The number of ejaculates necessary to collect to improve the semen quality will vary among stallions. Two consecutive ejaculates of similar quality should indicate that the stallion has voided the accumulated sperm.

SEMEN HANDLING

Raw semen is very fragile and should not be exposed to direct sunlight or sudden temperature changes. Once the stallion ejaculates, the semen should come into contact with a clean and warm container to prevent cold shock. The semen is filtered in line during ejaculation or filtered immediately after collection with nylon mesh or milk filter paper. This procedure is done to remove all extraneous particles and the gel fraction. Immediately after collection the raw semen should be placed in an incubator at 37°C prior to extension. The volume and color of the ejaculate should be recorded. In addition, the other physical characteristics of the ejaculate such as sperm movement, sperm concentration, and sperm morphology should be assessed from a small aliquot (1–2 ml) of raw semen that is removed prior to the addition of any diluents or extender. Once semen is diluted with a prewarmed extender it should be immediately removed from the incubator and cooled down to room temperature (20°C). An accurate assessment of the percentage of progressively motile sperm can only be done when the semen has been diluted to 25 million to 50 million sperm per milliliter.

PRESERVATION OF SEMEN

The processing of semen should be done according to the period of time that the semen is expected to remain viable. It is recommended to always place the semen in an appropriate extender even if it is going to be inseminated within a few minutes. There are several reasons why semen should be extended within a few (<5 minutes) after collection. A proper extender buffers pH changes of the raw semen, maintains the osmolality, provides energy and protein sources for sperm metabolism and membrane stabilization during thermal changes, reduces the detrimental effects of the seminal plasma, and provides antibacterial properties through the antibiotics. Semen can be preserved for varying periods of time. However, the state and the processing technique will be determined by the expected longevity of a given ejaculate. Fresh extended semen can be kept at 20°C, avoiding exposure to direct sunlight, without losing its fertilizing ability. If semen is intended to last 12 hours or more, either because the stallion will not be available or because the semen will be shipped, it should be slowly cooled and maintained at 4 to 8°C. If semen is to be stored longer than 72 hours, freezing is recommended.

It is extremely important to ensure that the extenders that are going to be in contact with the sperm are isothermic with the semen. In other words, extender that will be added to raw semen should be prewarmed. However, extender added to semen that has been cooled to room temperature should be also at room temperature. Failure to do so induces cold shock to the sperm, resulting in irreversible reduction of the progressive motility and membrane damage reducing the fertilizing potential.

FRESH SEMEN

Semen that is collected and used immediately or up to 6 hours after collection does not necessarily have to be cooled and in most cases can be diluted with prewarmed extender at ratios of 1:1 to 1:3, depending on raw semen concentration and ejaculate volume. Immediately after extension, the semen is removed from the incubator and can be cooled to room temperature (15–20°C) without loss of its fertilizing potential.

COOLED SEMEN

AI using cooled semen is still gaining popularity among breeders since more breed registries are accepting registrations of foals resulting from the use of this technology. Although there is a tremendous increase in its use there is also a tremendous variability in the results achieved. In order to minimize the variability in the results it is important that the handling and processing of the semen be standardized.

The semen handling and processing factors that could have the biggest impact of the quality of an insemination dose are (1) cooling rates and containers, (2) dilution rates, (3) and the insemination process.

Although there are several extenders that can be used for preserving semen in its liquid state it appears that most researchers and clinicians favor the use of nonfat, dried milk solids and glucose or the Kenney extender. This is perhaps due to its ease of preparation and handling, as well as its clarity making it easy to evaluate sperm motility.

Cooling Rates and Containers

Spermatozoa suspended in an extender could be rapidly cooled from 37° to 20°C, but require a slow cooling rate of –0.5° to –0.1°C per minute between 20° and 5°C to maximize spermatozoal viability. If sperm are cooled slowly, once they have reached 4° to 8°C, this temperature storage is superior to storage at 20°C. It appears that the ideal storage temperature range for maintaining motility and fertility of cooled equine semen preserved

for more than 12 hours in a Kenney type extender is between 4° and 6°C. However, Magistrini and associates found that a storage temperature of 15°C was superior to 10° or 4°C for maintaining spermatozoal motility when the extender does not contain milk solids. The current industry standard for preparing semen for shipping is to pack 1×10^9 progressively motile sperm in a nonfat dried milk (NFDM) glucose extender containing antibiotics, so that the final sperm concentration is 25 to 50 $\times$ 10^6 sperm/ml. Semen is slowly cooled, maintained, and stored at 4° to 8°C.

There are several containers currently available commercially that are designed for cooling and transport of equine semen. The Equitainer is the most widely used container for passive cooling and transporting of equine semen. Less expensive, disposable semen-transport containers are also available, such as the Equine Express or Expect-A-Foal. These containers are also passive-cooling transport devices, which provide variable rates of cooling. However, the big difference between the Equitainer and other semen transport systems is the effect of environmental temperature on the rate at which the semen is cooled or their ability to maintain their internal temperature. A study conducted to evaluate the effect of storage containers and environmental temperature suggested that sperm motility is adequately maintained in most commercial equine semen transport containers when the outside temperature is between 22°C and 37°C. However, environmental temperature of –20°C for 6 hours resulted in the reduction of progressive motility in most of the disposable containers. This study suggested that the Equitainer was a better container to isolate the semen from the effect of environmental temperatures.

Semen Extender and Dilution Rates

The majority of extenders used for fresh and cooled equine semen in North America are nonfat dried milk solids with glucose and antibiotics. Most of these extenders are variations of the original Kenney extender described in 1975. The variations of this extender are primarily a change in the sugar content or the type of antibiotic added. Two types of sugars are primarily used: glucose or sucrose or a combination of the two. Some of the countries in continental Europe such as France, Holland, and Germany use other types of extenders for routine cooling and shipping. French stallion farms ship semen in an ionic extender supplemented with phosphocaseinate, which does not need to be cooled. On the other hand, Dutch and German stallion semen is centrifuged and the sperm is resuspended in an egg yolk based extender for fresh or cool shipping.

The most common antibiotics include a combination of potassium penicillin and amikacin sulfate, gentocin, and ticarcillin. It is strongly recommended to avoid the use of polymyxin B because it has been shown to have a deleterious effect on sperm motility. The bacterial flora of the stallion and the effect of the antibiotic on the sperm motion characteristics for the individual stallion should be determined, because the most appropriate antibiotic(s) could vary between stallions.

Although the processing and handling of the sperm is tolerated well by the semen from the majority of the stallions, some stallions have a significant and unexplainable reduction of the semen quality when cooled and stored for over 12 hours. It appears that for some of the "problem" stallions, partial or total removal of the seminal plasma is important to maintain the percentage of progressively motile sperm. The ideal dilution rate at which sperm should be extended will vary according to the sperm concentration in the raw semen and the total number of progressively motile sperm. The author has found that semen with 100 million sperm should be diluted at a 1:3 ratio (semen: extender). Every increment of 50 million sperm/ml will increase the dilution rate by 1. So when the concentration of the ejaculate is between 450 million and 500 million per milliliter the dilution rate would be 1:10. Extending the semen using this method will result in a final sperm concentration of 25 million to 50 million per milliliter. The final volume to be shipped will be calculated so that a total of at least 1 billion sperm cells are sent to the mare.

The packaging of the semen when using the skim milk glucose extender should be performed under anaerobic conditions. Semen can be packaged in preloaded syringes, disposable baby bottle liners or whirl pack bags, provided that all the air is removed from the package prior to placing it in the container.

Insemination of Cooled Semen

A considerable amount of controversy exists regarding the number of insemination doses and the number of times that the mare should be inseminated. In the author's opinion there is no clear answer and it would depend on the quality of the stallion's semen and the reproductive history of the mare. If the semen is of good quality and the mare is a young fertile mare, she could be inseminated once or twice at 12- or 24-hour intervals. However, if the semen has poor quality it would be important to breed the mare as close to ovulation with the entire dose. On the other hand, if the semen is of good quality but the mare is a problem mare with a tendency to accumulate uterine fluid, she should be bred only once within 24 hours prior to ovulation. The combination of a problem mare bred with poor quality semen should be avoided because this will be a source of aggravation for the mare owner, the stallion owner, and the veterinarian. However, if there is no other option, these mares should be bred as close as possible to ovulation with all the semen that is available.

FROZEN SEMEN

In general the freezing process involves the collection of semen from the stallion, evaluation of the semen, dilution and centrifugation, and resuspension of the sperm in freezing extender. Unfortunately frozen-thawed sperm appear to have a shorter life than fresh or cooled sperm. This appears to be partially related to changes inflicted on the spermatozoa during the process of freezing and thawing. These changes, collectively called cryodamage, could be lethal if the sperm is rendered infertile or sub-

lethal if the change is reversible. Owing to apparent short lifespan of cryopreserved semen the insemination process with frozen semen requires more infrastructure and is more labor intensive than insemination with fresh semen and often results in reduced fertility compared to other breeding methods.

Facilities

To increase the pregnancy rates with frozen semen it is recommended to breed mares in a central location where the veterinarian is able to examine the mares on a regular basis and has proper equipment for thawing and evaluation of thawed semen. Because it is imperative to breed the mares close to ovulation, accuracy in the prediction of the time of ovulation is critical. Therefore, the availability of ultrasound equipment is required. In addition it is strongly suggested that the facility where the mares are inseminated be equipped with a liquid nitrogen tank for the storage of semen. Thawing, evaluating, and loading the semen requires an incubator, an accurate thermometer, a clean water bath, a good quality microscope, clean slides, coverslips, and a watch.

Pregnancy Rates with Frozen Semen

The success of a frozen semen program will depend on (i) The quality of the frozen-thawed semen; (ii) selection of the mares and stallions; and (iii) management of the mares during the breeding cycle.

Semen Quality

Currently about one fourth of the stallions whose semen is frozen will have pregnancy rates over 60% per cycle. Some of the remaining stallions, although they will have a lower pregnancy rate per cycle, can reach acceptable levels of fertility by the end of the season. The drawback is that the number of breeding cycles to achieve acceptable pregnancy rates is often too high or too expensive for the mare owner. Unfortunately, individuals performing the insemination rarely have control of the quality of the semen that must be used. Because frequently the semen quality is not very good, careful mare selection as well as excellent reproductive management are critical in determining the outcome of that breeding.

Thawing and Evaluation

Temperature at which the semen should be thawed depends on the type of straw in which the semen is packaged. Straws of 0.25 or 0.5 ml are generally thawed at 37° C for a minimum of 30 seconds. Care must be taken if multiple 0.5-ml straws are thawed at once to ensure that the straws will not adhere to each other while thawing, which will create an uneven thawing rate in the straws. Accuracy in the thawing time and temperature are critical when using thawing 0.5-ml straws at 75° C for 7 seconds. Semen could be also frozen in 2.5-, 4-, or 5-ml straws. Sperm packaged in these straws, regardless of volume, should be ideally thawed at 50° C for 45 seconds, although some individuals will thaw them at 37° C for 2 minutes.

There is no standard procedure or protocol to evaluate frozen semen. In addition, none of the tests routinely used to evaluate cryopreserved semen appear to correlate well with fertility. Furthermore, there are no set standards for the minimum quality of an insemination dose. Despite the lack of standardization, it is agreed by most laboratories freezing semen commercially that the minimum criteria required in an insemination dose are 30 to 35% progressively motile, 50% morphologically normal, and greater than 600 million total sperm per dose. Increasing the number of sperm to 800 million total increases the fertility in a linear way, but no further increase was detected when total sperm concentration reached over 800 million. Colorado investigators reported that stallion semen frozen in 0.5-ml straws that had 320 million progressively motile spermatozoa after thawing resulted in higher pregnancy rates compared to semen frozen in straws at lower concentrations. There is still no consensus on the minimum number of progressively motile sperm per dose that are needed to maximize fertility, and perhaps this is a stallion-dependent factor as has been reported for the bull. Vidament and associates have reported that 300 million sperm inseminated every 24 hours resulted in higher pregnancy rates compared to inseminations having 150 million sperm per dose. Semen frozen in The Netherlands is required to have a minimum of 300 million progressively motile and morphologically normal sperm per dose after thawing in order to be sold commercially.

Because the evaluation of post-thawing motility is a fairly subjective measure of quality and is also a poor predictor of fertility, it is recommended that veterinarians using frozen semen routinely evaluate the morphology of the sperm. This is particularly important when the pregnancy results are suboptimal despite apparent good motility.

Stallion Selection

The large variation among stallions' semen to tolerate the freezing and thawing process is poorly understood. Constituents of the seminal plasma or molecules on the sperm itself could be involved in the variability; however, it is important to realize that the selection criteria for stallions rarely, if ever, involves semen quality or fertility. Therefore, colts sired by stallions with inherent good semen quality are more likely to have good semen themselves. Semen from some of these stallions and their sons have consistently resulted in excellent post-thaw quality and pregnancy rates above 60% per cycle. The previous fertility of the stallion with fresh or chilled semen is not necessarily a good indicator of the stallion's fertility with frozen-thawed semen. In order to increase the chances of success it is advisable to inquire regarding the previous fertility of the stallion with frozen semen so that only stallions of proven success are used.

Mare Selection

The average number of cycles per pregnancy is higher for mares inseminated with frozen-thawed semen compared to natural cover or fresh artificial insemination. It is therefore critical that the mare owner is made aware of this potential reduction in fertility so that stallions and mares

with good fertility histories are selected. Even after careful stallion and mare selection proper client communication is imperative so that a nonpregnancy result after the first cycle will not discourage future breedings. Increasing the number of breeding cycles will undoubtedly result in higher pregnancy rates for mares by the end of the season.

Owing to changes in the horse industry, an increasing number of older maiden mares (>8 years), which have had a prolonged sport or show career, are being bred by artificial insemination. These mares and mares with histories of reproductive problems in general have significantly lower pregnancy rates, are potential candidates for disappointments, and are considered poor frozen semen candidates. Mare status has been shown to have a significant effect on the chances of a mare becoming pregnant. First cycle pregnancy rates with frozen semen for young maiden mares (younger than 7 years old) are around 65%; older maiden mares (over 8 years of age) 35%; barren mares 50%; and mares nursing a foal, 51%. Old maiden mares and those that have been barren for more than 2 years appear to have the poorest fertility. When only mares with excellent reproductive histories are inseminated with frozen semen, first cycle pregnancy rates of 100% on the young maiden mares and 75% in barren and foaling mares clearly indicate the importance of careful mare selection when breeding with frozen semen (Metcalf 1995).

Management of Mares

It is important to ensure that mares that are going to be bred with frozen-thawed semen are cycling regularly. Transitional heats should be avoided in order to increase the reliability of ovulation and reduce the use of semen. All mares should have at least a uterine culture and cytologic examination performed. These procedures can be obviated in young maidens (<6 years) with good perineal conformation. However, young maiden mares with evidence of fluid accumulation should also be cultured. A mare with a positive culture and cytologic findings should be infused in the uterus for at least 3 consecutive days when her cervix is dilated. Daily monitoring is necessary to ensure that no fluid accumulation is present in the uterus.

The availability of frozen semen is often limited to one or two doses per cycle or three to five for the entire breeding season. Therefore, it is important to maximize the use of the semen by making sure that the mares have a clean culture and cytologic test and by breeding mares once or no more than twice during one cycle. To achieve this, mares should be examined every other day or daily during the first days of the heat period and more intensively when close to ovulation. When the semen is of good quality every attempt should be made to breed the mares within 12 hours prior to ovulation. Mares that do not ovulate after the first insemination should be examined every 12 hours and inseminated with a second dose immediately after ovulation is detected if semen is available.

Timing of insemination. Sperm that have been frozen and thawed appear to have reduced longevity because of the membrane changes inflicted on them during the process of cryopreservation. Therefore, to maximize fertility, it becomes critical to try to breed the mares as close to ovulation as possible. Unfortunately, this is a more labor-intensive process for the veterinarian and more costly to the mare owners. In the author's experience, mares have a higher pregnancy rate when insemination is performed within 6 to 12 hours prior to ovulation. If mares have not ovulated within the first 12 hours after the AI, then a second dose should be inseminated immediately after ovulation has been detected on rectal examinations performed every 12 hours. In this author's experience fertility of mares that are bred only once after ovulation when rectal examinations are only performed every 12 hours is reduced by an additional 20% compared to those mares bred only once before or before and after ovulation. Although some experienced veterinarians have reported very high pregnancy rates when inseminating only once after ovulation, it seems that that to maximize pregnancy rates with single postovulation breedings, mares should be bred within 2 to 4 hours after ovulation. Because there is no ultrasonographic or palpable parameter to age the corpus luteum in a horse, it is necessary to examine mares at 2- to 4-hour intervals in order to be accurate.

An ovulatory inducing agent should always be used in order to reduce the number of examinations and to reduce the time from breeding to ovulation. Two products are currently available to induce ovulation in mares: human chorionic gonadotropin (hCG) and deslorelin, a GnRH analogue implant (Ovuplant). Both products are effective in inducing ovulation. Typically 2500 IU of hCG are given intravenously when the mare is displaying behavioral estrus, has a follicle of at least 35 mm, and has obvious and distinct edema of the endometrial folds. Ovulation in these mares occurs at 36 ± 17 hours. Because of the range in ovulation timing, mares treated with hCG should be palpated and evaluated with ultrasound every 6 to 8 hours after the injection. If the same criteria are followed to treat mares, Ovuplant induces a reliable ovulation in a narrower window (38 ± 2 hours) after treatment. Even when using an ovulatory inducing agent, the follicular and uterine texture should be evaluated closely and routinely by rectal and ultrasonographic examination in order to determine the ideal breeding time.

Availability and cost of semen is an important factor in determining the timing and number of doses to be used on a per cycle basis. It is encouraged that mare owners purchasing frozen semen be provided with a minimum of two doses per cycle.

Insemination technique. The number of straws per insemination and the thawing procedure should be performed following the instructions from the laboratory processing the semen. In general multiple (4–8) 0.5- or 0.25-ml straws are necessary for one dose and should be thawed at 37°C for at least 30 seconds. However, some laboratories will freeze one insemination dose on one 0.5-ml straw. When semen is frozen in 2.5-, 4-, or 5-ml straws commonly the processor will recommend one straw per insemination dose that should be thawed at 50°C for 45 seconds.

The straw(s) of thawed semen will be removed from the water bath and dried thoroughly. It can be more

practical to cut the straws that constitute an insemination dose at one end and place the entire volume of semen in a dry, warm clean tube, or directly into a warm syringe case. The semen should be evaluated microscopically prior to inseminating the mare. It is not recommended to add any type of extender to the thawed semen prior to insemination because of a high risk of causing an osmotic stress to the sperm. The semen should be slowly deposited into the uterus using a regular insemination pipette. Using the same syringe the pipette is flushed with air a couple of times. After the semen is in the uterus, the pipette is rinsed with 4 to 6 ml of a skim-milk glucose extender and the pipette again is flushed with air once or twice. It is important to perform these procedures slowly to increase the volume of semen deposited into the uterus.

The standard insemination technique is to place the semen in the uterine body. However, recently several groups have reported differences in pregnancy rates when mares were bred with reduced sperm numbers around the oviductal papilla. It seems that higher numbers of sperm reach the mare's oviduct when they are bred deep in the uterine horn ipsilateral to the dominant follicle, and therefore, veterinarians using frozen semen should consider inseminating deep in the uterine horn as an alternative to uterine body insemination.

Postbreeding therapies. The postinsemination examination is a crucial component of the insemination process and should be done not more than 12 hours after insemination. The purpose of this examination is to confirm that the mare has ovulated if she was bred prior to ovulation and to determine the presence of inflammatory exudates or fluid accumulations in the uterus regardless of the time of breeding. This is particularly important in old maidens, mares with delayed uterine clearance, and mares susceptible to uterine infections. Appropriate therapies such as uterine lavage, oxytocin injections, postbreeding antibiotic infusions, and Caslick's procedures should be performed. Blanket therapies such as uterine lavage or postinsemination infusions on all mares bred with frozen semen are not necessary. However, mares should be treated based on their clinical signs and previous reproductive history.

Allergic Reactions to Frozen Semen

There are a number of mares that accumulate excessive amounts of fluid or purulent material within a few hours after the insemination with fresh extended, cooled, or frozen semen. Some mare owners believe that some of these mares are allergic to certain stallions' semen or to components of the extender in which the sperm has been diluted. Several groups have investigated this "problem," and their data indicate that a slight amount of fluid accumulation in the uterus after insemination is a normal process. This reaction appears to be in response to the spermatozoa and does not depend on the type of extender or any of its components such as egg yolk, milk proteins, or glycerol.[1] In addition, no evidence of an allergic reaction has been detected in these mares. Small amounts of uterine fluid can be detected on most mares shortly after breeding and is a transient uterine inflammatory and a normal process in all mares. Seminal plasma appears to be a modulator and delays the physiologic uterine reaction to semen. Since the process of freezing semen involves the removal of most of the seminal plasma, this probably increases the transient inflammatory response in the mare. Small to moderate amounts of fluid are drained through the cervix within a few hours of breeding in the normal mare. Excess fluid accumulation is perhaps the result of poor cervical relaxation coupled with poor lymphatic drainage in the old maiden mare, or poor uterine contractility in mares susceptible to uterine infections. Mares that are in these categories should be routinely checked and a uterine lavage performed. In addition, oxytocin therapy should be implemented to aid in uterine contractility and drainage of the excess amount of fluid.

The number of mares bred artificially is increasing every year. AI in horses results in the birth of thousands of foals every year. Cooled transported and frozen semen breeding, unlike natural cover, does not allow for many errors in the handling of semen or flexibility in the timing of breeding. This results in an increase in labor for the veterinarian and cost for the mare owner that should be clearly discussed before starting the process. Careful selection of the mares and stallions will further increase the chances of success in an artificial breeding program.

Reference

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CHAPTER 6

Stallion Sexual Behavior Dysfunction

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INADEQUATE SEXUAL INTEREST AND AROUSAL; SLOW OR VARIABLE RESPONSE

Common complaints concerning inadequate sexual interest and arousal or slow or variable response include slow starting novice breeders, slow or sour experienced stallions, and specific aversions or preferences.[1] Certain genetic lines tend to be quiet or shy breeders under domestic in-hand conditions. A large portion of inadequate libido in stallions is believed to be the result of domestic rearing, training, or breeding conditions. Most conspicuously, stallion sexual behavior can be suppressed by adverse experience.[2] For example, stallions that have been disciplined for showing sexual interest in mares during their performance career, or are discouraged from showing spontaneous erection and masturbation, or are injudiciously or roughly handled during breeding under halter are at risk of libido and sexual behavior problems. When exposed to a mare for teasing, such stallions may simply stand quietly, may appear anxious and confused, or may savage the mare. Most stallions with experience-related libido problems respond well to behavior therapy alone or in combination with anxiolytic medication.[2] These stallions typically respond well to extended exposure to mares, either in direct contact (turned out together) or in adjacent paddocks or stalls. Some extremely inhibited stallions may not respond in the presence of humans. Once they are responding at pasture, they may require gradual introduction to human handling during sexual interaction with mares. Confident, respectful, patient, positive reinforcement–based handling greatly facilitates the transition to breeding in-hand. These stallions appear to respond favorably to reassurance for even small increments of improvement. Tolerance of minor misbehavior, rather than punishment, is often the most effective initial retraining strategy with low libido stallions. Once these stallions understand and gain confidence with the domestic breeding process, necessary breeding manners can be imposed without suppressing arousal. Some of these stallions appear to demonstrate specific preferences or aversions, which can be for certain mares, colors or breeds of mares, handlers, locations, time of day, etc. The anxiolytic diazepam (0.05 mg/kg slow IV 5 to 7 minutes before breeding) is useful in many such cases, as an adjunct to behavior modification.

For novice breeding stallions in transition from a race career, exposure to anabolic steroids and other medications can be a factor in low libido. Depending upon the duration of treatment and the season in which treatment was discontinued, it may take several months for endocrinology, testicles, or behavior to appear normal. Libido may be more affected than fertility, and vice versa.

Management, as it affects sociosexual environment, can be a significant influence on stallion behavior, endocrinology, and sperm production. For most stallions, sexual and aggressive behavior are subdued when kept together with other stallions and away from mares as compared to being in management when the stallion is away from other stallions and exposed to mares.[3,4]

Spontaneous erection and penile movements, common in all mammals, are known as "masturbation" in stallions.[5] Episodes of spontaneous erection and penile movements occur at approximately 90-minute intervals in all stallions, regardless of age, breed, housing, exercise, sociosexual environment, breeding status, or fertility. Although the penis typically becomes fully erect and the glans penis may fully flare, ejaculation rarely occurs. While most stallions can be easily distracted momentarily from progressing with an episode, it is extremely difficult and likely beyond humane care to significantly inhibit the number of episodes. Accordingly, genital injury as well as fear of humans and suppressed sexual behavior can result when, due to simple misunderstanding of these normal periodic penile erection and movements, aggressive attempts are made to physically inhibit or punish the behavior.

In practice, a small proportion of cases of slow breeding novice or experienced stallions appears to be hormone-related, with androgens on the low side of the normal range. Some cases obviously involve trying to breed outside the natural breeding season in stallions that are especially seasonal. These stallions will likely improve with management aimed at increasing exposure to mares and reduced exposure to other stallions. This will typically increase androgen levels and general confidence, as well as sexual interest and arousal. Gonadotropin-releasing hormone (GnRH) (50 micrograms subcutaneously 2 hours and again 1 hour before breeding) can be useful in boosting libido in stallions, particularly in those with low normal levels.[6] In rare cases when more rapid improvement is required to rescue a breeding career, treatment with testosterone can effectively "jump start" a slow novice without apparent significant adverse effects on spermatogenesis. Current recommendations are 0.1 to 0.2 mg/kg aqueous testosterone subcutaneously every other day for up to 2 weeks, with frequent assay of circulating testosterone not to exceed 4 ng/ml. As described in Chapter 1, stallion testicular function

apparently will respond to artificially increased day light, but the experience has been that if long-day recrudescence is advanced with artificial lighting, so will the following seasonal decline be advanced. In other words, it appears that the stallion has a fixed length of peak seasonal reproductive function. Effects of artificially advanced photoperiod on behavior apparently have not been characterized. In practice managers report that some low libido stallions that must breed in late winter months benefit from 16 hours of light beginning in late fall. The same schedule of lighting that is used to advance seasonal cycling for mares is used.

For diminished libido in an experienced stallion, whether gradual or sudden, changes in general health, nutritional condition, housing, handling, breeding conditions, and breeding schedule should be considered as potential contributing factors. Changes in semen quality and testicular size and consistency often indicate endocrine factors.

SPECIFIC ERECTION DYSFUNCTION

Libido-independent erection dysfunction appears to be relatively rare in stallions. The majority of cases are related to traumatic damage of the corpora cavernosa, resulting in insufficient or asymmetrical tumescence (lateral or ventral deviations) that impairs insertion. In some instances, penile injury appears to impair sensory or proprioceptive feedback from the penis, delaying coupling, organized thrusting, or ejaculation. Common causes include stallion ring injuries, phenothiazine-related paralyzed penis and paraphimosis, kick injuries, and self-serve breeding dummy accidents.

A peculiar and often confusing type of erection dysfunction involves the folding back of the penis within the prepuce as it becomes erect. The behavioral hallmark of this situation is a stallion that appears aroused and ready to mount, without a visible erection. The stallion may also appear uncomfortable or intermittently distracted, pinning the ears, kicking toward the groin, or stepping gingerly on the hind legs. Close observation reveals a rounded, full appearing prepuce, with the skin stretched taut. Resolution usually requires removing the stallion from the mare until the penis detumesces. Once the penis is fully withdrawn, application of a lubricating ointment to the prepuce facilitates subsequent normal protrusion. This situation tends to repeat occasionally over time with particular stallions, particularly in stallions with either profuse smegma production or in stallions in which the penis and sheath are dry from frequent cleansing.

MOUNTING AND THRUSTING DIFFICULTIES

A significant percentage of breeding dysfunction appears to involve neurologic or musculoskeletal problems that affect the stallion's ability to mount and thrust. Stallions vary considerably in the degree of musculoskeletal or neurologic deficit or discomfort that affects breeding performance or libido. Many such stallions can continue breeding with therapy aimed at reducing discomfort and accommodating disabilities during breeding, including adjustments to the breeding schedule aimed at reducing the total amount of work. A detailed discussion of musculoskeletal and neurologic problems in breeding stallions can be found in Martin and McDonnell, 2003.[7] Lameness problems that are not readily apparent in brief ground examinations can in some cases be more apparent during breeding. Direct observation by a breeding lameness specialist or review of video samples of successful and unsuccessful breedings, when possible, can be useful in identifying problems. Playback at various speeds, from very slow or frame-by-frame to fast-forward playback can often draw attention to a specific problem. Figure 6-1 illustrates two standard views we recommend for video analysis of mounting and thrusting difficulties. Stabilization of the video camera on a tripod is strongly recommended for most amateur videographers to obtain satisfactory views that enable meaningful evaluation.

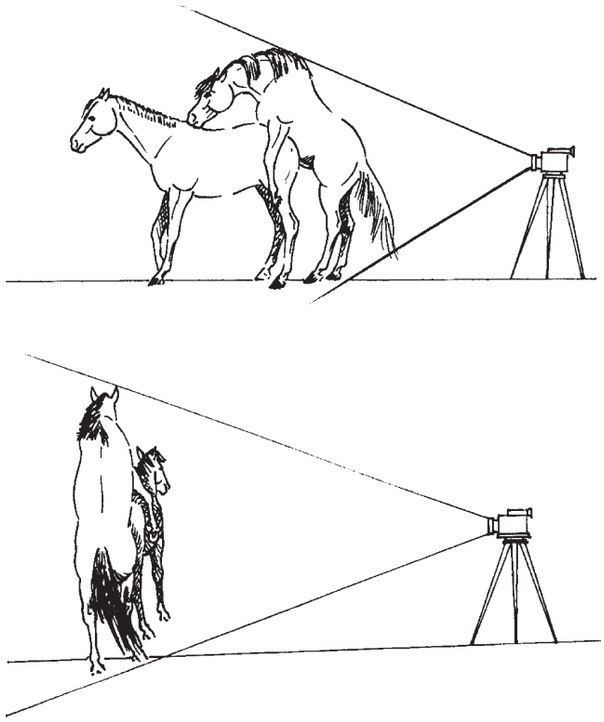

Fig. 6-1 Positioning of video camera on stable tripod for obtaining standard rear and side video views suitable for evaluation of mounting and thrusting difficulties in stallions.

Long-term treatment with oral phenylbutazone (2–3 mg/kg orally twice daily) can be used to maintain adequate comfort for breeding through a breeding season. Exercise programs carefully designed to improve overall stamina and to develop compensatory fitness, as well as weight management to reduce the effort of mounting and thrusting, can be beneficial in many instances. Management changes, including changes in breeding schedule, footing for breeding, housing and turn-out, diet, and handling in the breeding shed, all for optimal general attitude, libido, and performance of the individual stallion, can contribute significantly to extending the career of a stallion. Medications aimed at lowering the ejaculatory threshold can be useful in reducing the amount of work and resulting "wear and tear" on a busy stallion.[8] Imipramine hydrochloride (0.5–1.0 mg/kg orally 2 hours before breeding) can effectively reduce the ejaculatory

threshold. Similarly, xylazine at low levels enhances ability to ejaculate. Also, for stallions breeding by artificial insemination (AI), many can benefit from semen collection while standing on the ground as opposed to mounted.[9]

SPECIFIC EJACULATION DYSFUNCTION

Even though any libido, erection, or mounting and thrusting problem may result in failure to ejaculate, for some stallions the dysfunction seems to be specific to ejaculation. Specific ejaculation problems can include apparent failure of the neural ejaculatory apparatus, physical or psychological pain associated with ejaculation, and genital tract pathology. Common problems leading to ejaculatory delay or failure include occluded ampullae, either unilateral or bilateral, that appear painful and aortic iliac disease; psychogenic disruption of thrusting and premature dismount can result from mishandling, especially at the mouth while mounted. Similarly, stallions that are rushed to dismount, for example, to collect a dismount semen sample, may develop a pattern of dismounting before ejaculation. Therapy includes addressing as many contributing conditions as possible, as well as optimizing handling and breeding conditions and maximizing musculoskeletal fitness and libido to enhance the stallion's ability to overcome ejaculatory difficulty. Imipramine hydrochloride (0.5–1.0 mg/kg up to a total of 20 mg per horse orally 2 hours before breeding) can effectively reduce the ejaculatory threshold for most stallions.[2]

Ejaculation difficulty can be one of the first signs of neurologic disease in a breeding stallion, as well as a last lingering problem of neurologic disease. In addition to hind limb deficits, which are typically more apparent during mounting than during ground work, there are specific signs of pelvic neurologic deficits associated with ejaculatory dysfunction. These include poor anal tone when mounted, for example, anal protrusion, defecation, or flatus with each thrust; weak, irregular, or thready ejaculatory urethral contractions; atypical and variable ejaculatory pattern, for example, sperm poor or gel fraction before sperm rich fraction; wide fluctuations in semen characteristics, including sperm concentration and pH likely reflecting variable contributions from the ampullae and accessory sex glands.

For stallions using a dummy mount for frequent semen collection, a common factor apparently causing or contributing to mounting and thrusting difficulties, as well as specific ejaculatory dysfunction, is poor dummy fit.[8] Our experience is that most problems are related to mounting a dummy that is too low. This may lead to the stallion's advancing up the near side of the dummy rather than remaining squarely behind the dummy. This results in thrusting with the lower back bent rather than straight. Stallions in this condition have been observed to develop sore backs and retarded ejaculation or anejaculation. Raising the dummy mount usually helps to keep the stallion "squared up" and the back straight in a physiologic copulatory position during thrusting. Use of a less bulky model artificial vagina, such as the Missouri model as opposed to a Colorado model, and use of a wedged dummy mount (cut away at the rear to enable a more physiologic positioning of the artificial vagina, for example Breeders Choice, Aubrey Texas, http://www.breederschoiceonline.com/phantoms.asp), typically further enhance the ability of the stallion to maintain a good "squared up" copulatory position.

PHARMACOLOGICALLY INDUCED EJACULATION

Ejaculation can be induced *ex copula* with various pharmacologics.[10] Although none of the protocols developed to date are near 100% reliable, the method can be useful in extending the breeding career of disabled stallions. The most reliable regimen for most stallions is pretreatment with oral imipramine (2.2 mg/kg PO) followed 2 hours later by xylazine (0.4 to 0.6 mg/kg IV).

ROWDY BREEDING BEHAVIOR

Rowdy, misbehaved breeding stallions in most cases represent a human-animal interaction problem and the manner in which stallions are given limited, concentrated interaction with mares. Most rowdy stallions can be handled safely for breeding with retraining in combination with simple modifications of facilities to minimize accidents. Even strong, vigorous, and misbehaved stallions can be brought under control using consistent positive and negative reinforcement, with very little or no severe punishment.[11] Retraining can be done in a safe and systematic manner without abuse or commotion, usually within a few brief sessions. Some of the most challenging rowdy stallions may benefit from vigorous exercise under saddle or ground work, immediately before breeding. Exercise not only fatigues and mellows the stallion, but also establishes a pattern of the stallion's taking direction from a handler. For similar reasons, when retraining a stallion in the breeding shed, we find it helpful to follow an intensive schedule, with up to several breedings per day. With fatigue and reduced urgency to breed, many stallions seem more able to abide direction and learn the routine. With repetition for schooling over a short period of time, many rowdy stallions seem to more readily understand the routine.

Tranquilization of breeding stallions is generally not recommended. It is difficult to achieve levels of sedation that improve controllability without compromising musculoskeletal stability or ejaculatory function. Tranquilizing agents commonly used in stallions, such as xylazine or detomidine, can both facilitate and inhibit erection and ejaculation, depending on levels. Acepromazine is not recommended for use in stallions for any purpose, due to its association with paralyzed penis.

HYPERACTIVE OR FRENZIED BEHAVIOR

Distinct from simple rowdiness, some stallions are hyperactive or even "frenzied." The hyperactive behavior can be either year-round, or more typically, greater during the breeding season. Some will spend nearly their entire time budget frantically "climbing the walls," vocalizing (screaming), or frantically running a fence line. The behavior may include stereotypic weaving, pawing, pacing, or circling. Generally, frenzied breeding stallions

can benefit from more roughage and less grain in the diet, organized physical work and pasture exercise, and consistent housing in a quiet area. Careful observation (particularly video surveillance) can be useful in identifying environmental conditions and events that set off episodes or tend to quiet a stallion. In extreme cases, pasturing directly with mares can effectively quiet or sensibly occupy a frenzied stallion. L-Tryptophan supplementation (1–2 grams twice daily in feed) can have a calming effect on such stallions. Tranquilization for this purpose is not recommended in breeding stallions because of risk of paralyzed penis and paraphimosis.

SELF-MUTILATION

Self-mutilation, while not unique to stallions, is a severe and relatively uncommon fertility-limiting and life-threatening problem. It typically takes the form self-biting of the flank, chest, or limbs, with violent spinning, kicking, and vocalization. Self-mutilation in horses appears to occur in two distinct forms. One form appears to be a severe reaction to irritation or pain, and would be similar in males and females. The self-biting is typically targeted toward the site of discomfort. Another form occurs in males and is reminiscent of stallion inter-male aggression. Sniffing, nipping, and frank biting behavior is targeted at the typically inter-male sites of aggression—the groin, flank, knees, chest, and hocks. The sequence of the behavior follows closely to that of two males fighting, with sniffing and nipping of the groin, vocalization, stamping with a foreleg, kicking out with a hind leg, and then taking occasional larger bites. Episodes often appear to be stimulated by sight, sound, or smell (feces or oily residues) of another stallion. For some stallions, episodes are set off by sniffing their own excrement or oily residues on stall walls or doorways. Current recommendations are to (1) physically protect the stallion from injury by padding walls or limbs, blanketing, and muzzling as effective, (2) aggressively evaluate the housing and social environment to identify exacerbating and ameliorating conditions that may be manipulated for greatest relief, (3) reduce concentrates and increase grass and hay in the diet to increase feeding time and eliminate highly palatable meals (feeding tends to distract and occupy the stallion; concentrate meals tend to increase stereotypic behavior), (4) apply odor-masking agents (Vicks VapoRub or Acclimate) around the nares, and (5) provide as much organized exercise as possible, also to distract the stallion. Housing in a large paddock without solid objects against which to contact kicks may reduce the risk of limb injury.

SAVAGE STALLIONS

Quite distinct from generally or sexually rowdy or unruly stallions, and much less common, are the truly savage stallions. Truly savage stallions are those that attack humans, without apparent provocation, and with the apparent intent to kill. Specific behaviors involve charging with head lowered and mouth opened; grasping onto the shoulder, belt, or limb; and shaking, tossing, and stomping the victim. The attacks are typically intermittent, unprovoked, and unpredictable. Savage stallions are not necessarily high libido or otherwise aggressive stallions, and do not usually respond to violent discipline. These stallions are often described as otherwise especially docile and compliant. Managers may unwittingly attribute the attack to a particular person or circumstance. In practice, our recommendation for stallions that have savagely attacked has been to either euthanize or to manage under "bull stud" conditions, where the stallion is barred from direct contact with humans.

RESIDUAL STALLION-LIKE BEHAVIOR IN GELDINGS

Castration, regardless of the age or previous sexual experience, does not always eliminate stallion-like behavior. If given the opportunity, as many as half to two thirds of geldings will show stallion-like behavior to mares, many will herd mares, and some will even mount and appear to breed. Similarly, although castration does tend to "mellow" most horses, it does not eliminate general misbehavior. Traditional behavior modification is usually much more effective in controlling sexual and aggressive behavior in a gelding under saddle or in-hand than it is with an intact stallion. Also, treatment aimed at quieting sexual and aggressive behavior, such as progesterone (for example, altrenogest 50–75 mg orally daily), is typically more effective in geldings than in intact stallions or cryptorchid "geldings." Elimination of stallion-like herding and teasing at pasture is difficult. Pasturing separate from mares is often the only suitable management.

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CHAPTER 7

Clinical Reproductive Anatomy and Physiology of the Mare

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The dynamic functions of the ovaries, uterus, embryo, and fetus are beginning to be fully appreciated as mares are evaluated during different stages of the estrous cycle and pregnancy and during various seasons. Anomalies, injuries and age-related changes may occur in mares, necessitating the use of diagnostic, prognostic, and therapeutic procedures, which require a thorough knowledge of equine anatomy. Several references, including the corresponding chapter in the first edition of this text, are currently available and provide a comprehensive discussion of reproduction in the mare, complementing the information presented in this text.[1–4]

REPRODUCTIVE ANATOMY

The organs involved in reproductive function are not only physiologically but also morphologically dynamic. The reproductive tract in the mare consists of the ovaries, uterine tubes, uterine horns, uterine body, cervix, vagina, vestibule, and vulva. These are the organs that produce the oocyte, facilitate its fertilization, provide an environment for embryonic and fetal development, and transport the fetus from the maternal to the external environment. They cannot perform these functions without interacting with other organ systems. The normal development of the reproductive tract of the mare, as well as the physiology of the mare's estrous cycle (upon which there are seasonal influences), depend on the neural and endocrine functions of the epiphysis (pineal gland) and hypothalamus (including the hypophysis or pituitary gland). The interactions of the epiphysis and hypophysis with other structures within the nervous and endocrine systems, the reproductive tract, and the mammary glands affect sexual behavior, pregnancy, foaling, and lactation.[4] Anatomy textbooks by Sisson and Grossman; Nickel, Schummer, and Seiferle; and Dyce, Sack, and Wensing are the foundations of the anatomic knowledge of many veterinarians.[5–7] Every effort will be made to remain consistent with their descriptions as well as with the nomenclature approved by the *Nomina Anatomica Veterinaria* (NAV) and the *Illustrated Veterinary Anatomical Nomenclature*.[8,9] Unless specifically noted, all anatomic discussions will pertain to the postpuberal female.

The Reproductive Tract of the Mare

The female reproductive tract consists of paired ovaries, uterine tubes, and uterine horns contiguous with a uterine body and cervix, vagina, vestibule, and vulva. These organs, with the exception of the ovaries, are collectively termed the tubular genitalia. There is a continuous lumen from the cranial end of each uterine tube and uterine horn, via the uterine body and cervix, vagina, and vestibule to the labia of the vulva. The tubular genitalia provide a connection from the peritoneal cavity to the external environment. For further details, see the references at the end of this chapter.

Suspensory Ligaments

Structure and function. Bilaterally the ovaries, uterine tubes, and uterus are suspended from the lateral sublumbar and pelvic walls by the double-layered peritoneal folds referred to as the broad ligaments, divided into three anatomically nondelineated portions: the mesovarium (attached to the ovary), mesosalpinx (arising from the lateral surface of the mesovarium), and mesometrium (attached to the uterus and a portion of the cranial vagina) (Figs. 7-1 to 7-3).[1,4–7,10] The cranial or free border of the mesovarium is the suspensory ligament of the ovary, which attaches in the sublumbar region.[8,9,11] The ovarian bursa is a duplication of the broad ligament between the ovary and the uterine horn.[4]

The broad ligaments allow for functional as well as physical attachment of the reproductive tract to the rest of the body. They provide an avenue for arteries, veins, lymphatic vessels, and nerves, which supply the ovaries, uterine tubes, and uterus. These ligaments also contain loose adipose and collagenous tissues as well as smooth muscle that is continuous with the outer longitudinal muscle layer in the uterine tube and uterus. The broad ligaments are also dynamic structures. During gestation, the vessels and smooth muscle mass enlarge in the broad ligaments, resulting in an apparent thickening. This suspensory apparatus allows for movement of the ovaries, uterine tubes, and uterus. Embryologically, peritoneal pouches form between the suspensory ligaments of the rectum, uterus, and urinary bladder and the roof and the

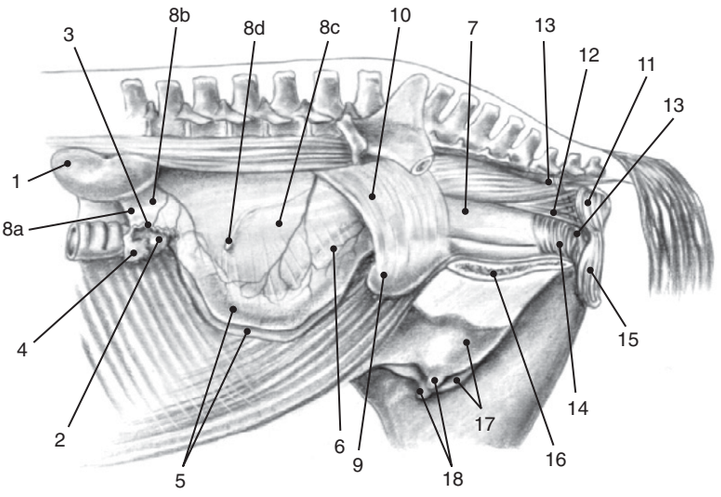

Fig. 7-1 Left lateral view of the urogenital apparatus of the mare. 1, Left kidney; 2, left ovary; 3, left uterine tube with ampulla and isthmus; 4, infundibulum of the left uterine tube; 5, left and right uterine horns; 6, uterine body; 7, vagina; 8, broad ligament (8a, mesovarium; 8b, mesosalpinx; 8c, mesometrium; 8d, round ligament of the uterus); 9, urinary bladder; 10, lateral ligament of the urinary bladder; 11, m. external anal sphincter; 12, m. levator ani; 13, m. retractor clitoridis; 14, m. constrictor vestibuli; 15, m. constrictor vulvae; 16, pelvic symphysis; 17, mammary glands; 18, papillae (teats).

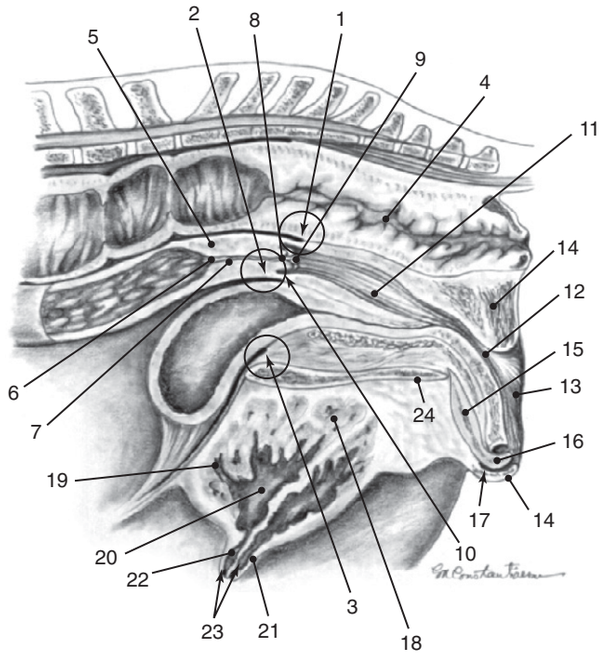

Fig. 7-2 Median section through the pelvic viscera in the mare. 1, Rectogenital peritoneal pouch; 2, vesicogenital peritoneal pouch; 3, pubovesical peritoneal pouch; 4, rectum; 5, uterine cervix; 6, internal uterine ostium; 7, cervical canal; 8, external uterine ostium; 9, vaginal portion of uterine cervix; 10, vaginal fornix; 11, vagina; 12, vestibule; 13, vulva; 14, m. constrictor vulvae; 15, corpus clitoridis; 16, glans clitoridis; 17, fossa clitoridis; 18, glandular tissue of mammary gland; 19, lactiferous duct; 20, glandular part of lactiferous sinus; 21, papilla (teat); 22, papillary part of lactiferous sinus; 23, papillary ducts; 24, pelvic symphysis.

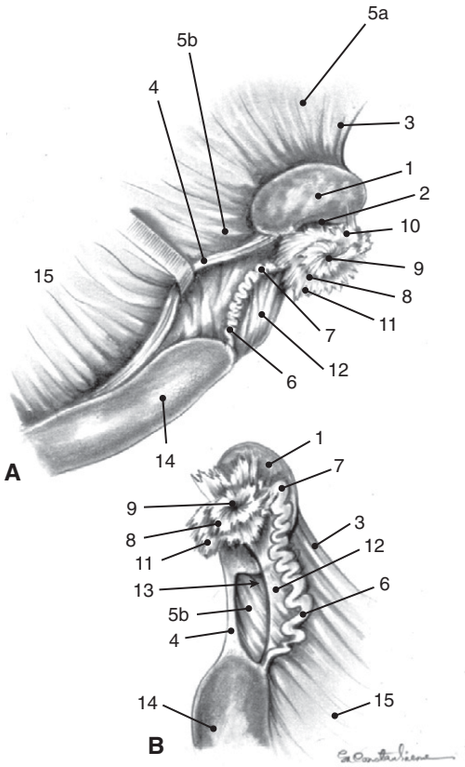

Fig. 7-3 Left ovary, uterine tube and associated structures. **A,** Medial view; **B,** ventral view. 1, Ovary; 2, ovulation fossa; 3, suspensory ligament of the ovary; 4, proper ligament of the ovary; 5, mesovarium (5a, proximal mesovarium; 5b, distal mesovarium); 6, isthmus of uterine tube; 7, ampulla of uterine tube; 8, infundibulum of uterine tube; 9, abdominal ostium of uterine tube; 10, ovarian fimbriae; 11, tubal fimbriae; 12, mesosalpinx; 13, ovarian bursa; 14, uterine horn; 15, mesovarium.

floor of the pelvic cavity, respectively. Cranially, these pouches open into the peritoneal cavity, and they end caudally in cul-de-sacs in the pelvic cavity against the pelvic diaphragm. The rectogenital pouch is formed by reflection of the peritoneum from the rectum onto the vagina. The reflection of peritoneum from the urinary bladder onto the vagina forms the vesicogenital pouch (Fig. 7-2).[1,4,7–11]

Clinical aspects. The anatomy of the broad ligaments can be clinically relevant in several situations. Exterior-

ization of the uterine horns or ovaries during surgical procedures is limited by the suspensory ligaments. The mesovarium is located for écrasement and ligation during ovariectomy, and in standing animals, local anesthetics are applied to the mesovarium prior to performing these procedures (Figs. 7-1 and 7-3).[12] As the vagina is rarely involved in uterine torsion in the mare (in contrast to the cow), transrectal palpation of the broad ligaments is necessary for diagnosis of uterine torsion and determination of the direction of torsion. In cases of uterine and ovarian arterial rupture, the mesometrium may contain the hemorrhage, or bleeding may dissect the broad ligament resulting in its rupture and exsanguination of the mare. Hematomas can be found in the mesometrium during routine postpartum examinations, and they may or may not be associated with clinical signs. Rupture of hematomas several weeks following parturition has been recorded.[4] It is important to remember that the anatomic boundaries of the rectogenital and vesicogenital pouches are variable and that this anatomic variability can be of clinical significance when performing an ovariectomy via a vaginal approach (colpotomy) or in assessing whether contamination of the peritoneal cavity has occurred following a vaginal or rectal injury (Fig. 7-2).[1,4,5,12]

The Ovaries

As in other domestic mammals, the ovaries perform exocrine (gametogenic) and endocrine (hormone production) functions in the mare. Exocrine function of the ovaries justifies the existence of the tubular genitalia and allows for the ovaries' hormonal integration and control of the reproductive tract.[1,3,4]

Location. The ovaries are traditionally the landmarks for transrectal palpation of the reproductive tract in mares. The ovaries and uterine tubes are the most cranial structures of the reproductive tract and may be found as far cranially as the third lumbar vertebra or as far caudally as the fifth lumbar vertebra. They are not as freely moveable as cows' ovaries, and, in nonpregnant animals or very early in gestation, they are often located 5 to 10 cm directly cranial to the upper third of the ipsilateral ileal shaft in the sublumbar region. The kidney lies cranial to the ipsilateral ovary by a distance ranging from 5 to 30 cm with the left kidney generally closer to the corresponding ovary. The left ovary is normally situated 2 to 3 cm further caudad than the right. The ovaries may be in contact with the corresponding uterine horn or may be separated by a distance of up to 5 cm. In circumstances in which the uterine horn is readily located, this anatomic relationship may be helpful in locating the ipsilateral ovary (see Figs. 7-1 and 7-3). As discussed previously, the mesovarium allows for variable positions of the ovaries depending on the stage of estrous cycle or pregnancy, seasonal influences, and the location of other abdominal organs. During pregnancy, the ovaries are frequently found pulled cranioventrally and medially. The ovaries frequently lie on top of the intestinal mass and are in contact with the dorsal abdominal wall; however, they may be found among the adjacent viscera.[1,4,5]

External structure. The mare's ovaries are reniform in shape and, in mares of light breeds, range from 5 to 8 cm in length, 2 to 5 cm in width, and 3 to 5 cm in height during the ovulatory season. Ovarian weight varies with age and stage of the estrous cycle, ranging from 30 to 120 g per ovary. The ventral (free) border of the ovary is concave and is indented by the ovulation fossa (ovarian fossa; NAV), which is an anatomic structure specific for the mare (Fig. 7-3).[1,3,4] When the intestinal viscera are removed and the reproductive tract is suspended by the broad ligaments, the ovaries are described as having medial and lateral surfaces, dorsal and ventral borders, and cranial (tubal) and caudal (uterine) extremities or poles. The dorsal (attached) border is convex, and, since this is where the mesovarium attaches and where vessels and nerves enter the ovary, it is correctly termed the hilus of the ovary. The ventral (free) border of the ovary is concave and is indented by the ovulation fossa (Fig. 7-3).[1,4,5,8–11] The caudal or uterine extremity of the ovary is attached to the uterus near the tip of the uterine horn by the proper ligament of the ovary (round ligament of the ovary or utero-ovarian ligament), which is a band of smooth muscle within the broad ligament (Figs. 7-1 to 7-3). In the embryo, this structure is continuous with the round ligament of the uterus and is homologous to the proper ligament of the testis in the male (part of the remnant of the gubernaculum testis).[1,4–6,8–10] Additional details regarding the histology of the external surfaces of the ovary are covered in the corresponding chapter of the first edition of this text.[4]

Internal structure and function. In most domestic mammals the ovaries consist of a peripheral parenchymatous zone (cortex), containing various stages of follicular and luteal gland development, surrounding a central vascular zone (medulla), comprising collagenous connective tissue rich in blood vessels. In the mare, the central parenchymatous zone, which surfaces at the ovulation fossa, is surrounded by the vascular zone.[1,4–7,11] Development of this unusual arrangement of the zona parenchymatosa and zona vasculosa in the mare will be discussed later.

The primary functions of the ovary are oogenesis (production of female gametes, oocytes, or ova) and steroidogenesis (production of estradiol and progesterone). During early gestation, the primordial germ cells arise from the epithelium of the embryonic yolk sac and migrate through the developing mesentery to the gonadal ridge (ovarian anlage) in its position contiguous with the mesonephros. Mitosis and meiosis of the oogonia do not occur beyond the sixth month of gestation and do not commence again until after puberty. There is substantial atresia of oocytes during fetal life, with only a portion surviving to parturition.[1,4,7]

The structural and functional unit of the ovary is the follicle. A primary oocyte surrounded by a single, flattened cell layer is a primordial follicle. There is an intact basal lamina separating the single layer of what will become granulosa cells from the adjacent stromal tissue, which eventually develops into the theca folliculi (theca cells). Mares are born with a finite pool of primordial follicles. It has been estimated that there is a reserve pool of approximately 36,000 primordial follicles in the mare (in contrast to over 120,000 primordial follicles in the cow). At any time during the ovulatory season, 100 (versus 300 in the cow) primordial follicles have left the reserve pool

and have entered the active pool of follicles and are undergoing growth and differentiation (folliculogenesis). At this point in their development, the follicles are called primary follicles. Once a primordial follicle joins this pool, it is destined to become atretic or ovulate. The oocyte grows in size and the zona pellucida is formed. The cells surrounding the oocyte differentiate into granulosa cells. A primary follicle is transformed to a secondary follicle when the single layer of granulosa cells differentiates into several layers. Primary and secondary follicles have traditionally been described as preantral follicles.[1,4]

Fluid exuded from granulosa cells of secondary follicles coalesces to form an antrum, and the follicle is transformed into a tertiary or antral follicle, which is primarily estrogenic. The wall of the follicle is formed by granulosa and theca cells. In the mare, follicles approximately 0.3 mm in diameter begin to form antrums, and these antrums may reach a size of 35 to 50 mm with follicular walls 5 to 6 mm in thickness. Studies in mares have estimated that it may take as long as two estrous cycles (42 days) for a follicle to grow from 0.1 mm to 1 mm. Other research has suggested that in cows the period required for a primary follicle to develop into a preovulatory follicle may be as long as 60 days. In mares, 50 to 75% of follicles greater than 1 mm in diameter present at any time are undergoing atresia, which is irreversible. Atretic follicles demonstrate degenerative changes in the oocyte and follicular epithelium. Fibroblastic remodeling of the remnants of large follicles results in scar tissue (corpus fibrosum atreticum).[1,4]

During each estrous cycle there may be up to two waves of follicular growth during which several follicles attain diameters greater than 15 mm. Usually one follicle ovulates from the primary wave, but occasionally there can be multiple ovulations or ovulation(s) from the secondary wave, as well.[1,3] The small number of follicles that ovulate migrate toward the ovarian fossa as their diameter increases, and they soften as they get closer to evacuation. Granulosa cells cease to divide a few days prior to release of the ovum. Immediately prior to follicle evacuation, granulosa cells undergo further differentiation, theca cells begin to degenerate, and there is a thinning of the follicular wall toward the exclusive site of ovulation in the mare, the ovulation fossa (Fig. 7-3).[1,3–6] At the time of ovulation, the first meiotic division is complete, and a secondary oocyte (arrested in metaphase II) is released.

A short time following ovulation, folds of stromal tissue that are accompanied by distended blood vessels, are formed by the collapsed inner wall of the follicle. Within 24 hours, granulosa cells have begun to undergo further growth and differentiation and are beginning to secrete progesterone. These changes to the granulosa cells constitute the process commonly referred to as luteinization. Proliferating capillaries accompanied by hypertrophied fibroblasts intermingle with luteinizing granulosa cells and replace degenerating theca cells. Nine days after the formation of the luteal gland, two types of luteal cells are evident, large and small. Eighty-five percent of the luteal cell population is composed of large luteal cells, which are almost four times larger than they were at the time of ovulation. The small cells are not derived from theca cells, as in other species, but are most likely granulosa cells in transition to becoming large luteal cells. By the twelfth day of the luteal phase in nonpregnant mares, the large luteal cells have decreased in size and in number. At the time of the next ovulation following luteal regression, the large luteal cells have undergone degeneration and the corpus albicans has been formed.[1,4]

Luteal glands have two distinct appearances when examined by ultrasonography (Fig. 7-4). Approximately 50% of the luteal structures contain a central blood clot that occupies greater than 10% of the cross-sectional area and progress through a corpus hemorrhagicum stage during formation of the mature corpus luteum (CL; Fig. 7-4, *A*). The central clots remain during the life of the CL but decrease in size and become more organized. The other 50% of ovulations result in formation of a solid or uniformly echogenic CL (Fig. 7-4, *B*). There is no significant difference in progesterone secretion by these two differently appearing luteal structures.[1,3,4] In nonpregnant mares, the luteal gland begins to regress approximately 12 to 14 days following ovulation and begins to form a corpus albicans by the start of the following estrus.[1,4]

Prepuberal ovarian development. At birth the ovaries are oval rather than reniform in shape; cuboidal germinal epithelial cells cover their ventral (free) surface, and the ovarian fossa has not yet formed. The fetal gonads at their greatest size during gestation are ten times larger than the foal's ovaries at parturition. By 3 months of age, the cortex (parenchymatous zone) is located beneath the infundibulum suggesting the future site of ovulation. The parenchymatous zone becomes invaginated into the vascular zone causing the ovarian poles to be drawn toward each other. In this manner, the unique anatomic relationship between the zona parenchymatosa and zona vasculosa seen in the ovary of the postpubertal mare begins to develop. As early as 5 to 7 months of age and certainly by puberty, the ovaries are kidney-shaped with a definite ovulation fossa.[1,4,11]

Clinical aspects. Transrectal examination of the ovaries by palpation and ultrasonography is one of the more commonly performed diagnostic procedures in mares. Confidence in locating the ovaries and identifying the normal, pertinent structures allows efficient determination of the mare's suitability for breeding and an accurate diagnosis of ovarian pathology, as well as a decreased risk of rectal injuries. Larger follicles can frequently be palpated as fluctuant structures; however, as the mare always ovulates from the ovulation fossa, smaller preovulatory follicles may sometimes be difficult to assess by transrectal palpation alone.[3,13–15] This certainly may be the case in younger or smaller mares that exhibit some resistance to the procedure. Although recent ovulations can be appreciated by palpation, luteal structures are often difficult to detect, especially if the mare's estrous cycle has not been followed closely by serial palpations. Transrectal ultrasonic imaging of the mare's ovaries allows visualization of follicles, ovulations (during estrus and diestrus), ovarian hematomas, and CLs (Fig. 7-4). In addition, ultrasonography has made possible the detection of multiple ovulations, anovulatory hemorrhagic structures, ovarian abscesses, and tumors.[1,3,4,13–16] Using

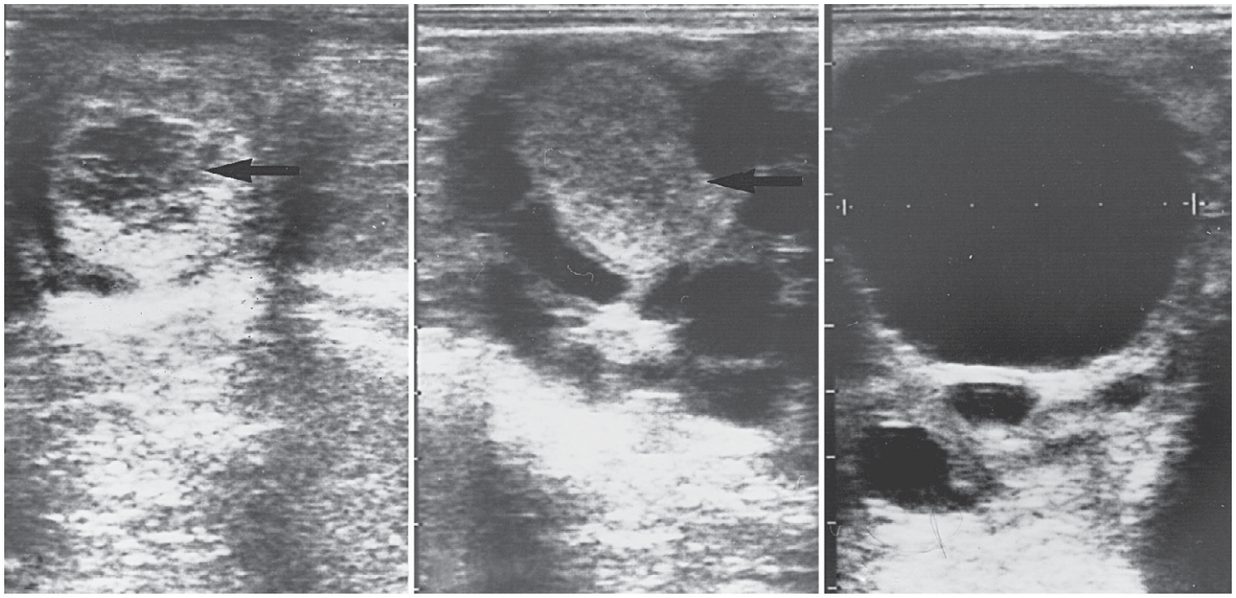

Fig. 7-4 Ultrasonographic appearance of ovarian structures. **A,** Solid arrow points to a centrally nonechogenic CL. **B,** Solid arrow points to uniformly echogenic CL surrounded by small follicular (>50 mm) structures. **C,** Calipers mark a large preovulatory follicle observed in a mare late during the ovulatory season. (Photographs courtesy of Mr. Howard Wilson.)

ultrasonography, it has been documented that there is an increased incidence of left-sided ovulations in maiden mares and that there is no predilection for nocturnal ovulations, as was previously thought.[1] The routine use of cooled, transported, and frozen semen and the performance of embryo and oocyte collection and transfer procedures has necessitated refinement of the ability to pinpoint the time of ovulation using ultrasonic imaging of the ovaries.[4,17–19] Irregularly shaped or flattened follicles with echogenic spots in the follicular fluid, as well as decreased follicular pressure and separation and hyperechogenicity of the follicular wall, are some of the changes noted just prior to ovulation.[3,4,15,20]

Knowledge of normal ovarian morphology and development can be helpful in the diagnosis of a variety of pathologic conditions and is critical for safe and successful oocyte recovery.[1,3,4,17–19] Not infrequently, mares are palpated, and both ovaries are found to be small and inactive. Seasonal influences account for the majority of these cases, but a history of anabolic steroid administration or chromosomal abnormalities (leading to gonadal dysgenesis) can account for some of these cases.[4,13,14] Although subject to some debate, it is generally thought that mares do not experience cystic follicular degeneration, as do other species.[4,13,16] Large ovarian follicles, which might be mistaken for follicular cysts, are often found during the early spring or late fall due to seasonal influences. Mares may develop epithelial inclusion cysts (fossa cysts), which can invade the ovarian stroma, and these cystic structures can potentially be a cause of infertility in older mares. Older mares are also more likely to have abnormal ovulations, which result in defective oocytes and embryos.[4,13–16,21] Ovarian hematomas are frequent occurrences, and although the ovary may be greatly enlarged, the mare continues normal ovarian activity. A number of different types of ovarian tumors have been observed in mares, including granulosa-theca cell tumors, dysgerminomas, teratomas, and cystadenomas.[1,3,4,14,16,21] The most common of these is the granulosa or granulosa-theca cell tumor. These tumors can be very large, and affected mares can exhibit several different patterns of abnormal behavior. The contralateral ovary is usually small and inactive, and the ovulation fossa is normally not palpable. However, exceptions have been observed, and these cases may represent the clinical presentation early in the course of tumor development.[4] It has been reported that excessive adrenocortical tissue can occur on the surface of the ovary and invade adjacent tissues. This most likely represents a pathologic manifestation of the adrenocortical nodules frequently seen in mares.[4,16]

The Uterine Tubes

The uterine tubes are paired structures, each associated with an ovary and uterine horn. Known by a variety of names including oviducts, salpinges, and fallopian tubes, these structures are the initial segment of the tubular genital tract. Like the uterus and vagina, they are derived embryologically from the paramesonephric ducts (müllerian ducts) and perform a variety of functions.[1,4,7]

External structure and location. The external surface of the uterine tube is covered by visceral peritoneum or serosa that is an extension of the squamous epithelium (mesothelium) of the mesosalpinx. This epithelium is contiguous with the mucosal lining of the infundibular portion of the uterine tube.[1,4] When dissected free from the mesosalpinx, each uterine tube, which is located

adjacent to the lateral surface of the ipsilateral ovary, is 20 to 30 cm in length and is divided into three segments—the infundibulum, the ampulla, and the isthmus (Figs. 7-1, 7-3, and 7-5).The funnel-shaped infundibulum has irregular fimbriae along its margins and a centrally located abdominal ostium. The tortuous ampulla, which is of a similar diameter to the abdominal ostium of the infundibulum, gradually decreases in diameter and extends from the infundibulum to a poorly defined point in proximity to the lateral surface of the uterine extremity of the ovary, where the isthmus begins. The isthmus is less tortuous, and its terminus, the uterotubal (tubouterine) junction, consists of a small papilla projecting into the uterine lumen. The oviductal lumen communicates with the lumen of the uterine horn through the uterine ostium in the papilla.[1,4,7–9,11]

Internal structure and function. There is a divergence in the internal structure of the portions of the oviduct and this structural alteration suggests that the primary functions of the uterine tube vary from the ovarian to the uterine extremities. The muscular layer (myosalpinx or tunica muscularis) consists primarily of an inner layer of circularly arranged muscle fibers covered by an outer layer of longitudinally oriented fibers. These longitudinally arranged fibers are continuous with the smooth muscle present in the mesosalpinx. There are very few muscle fibers in the fimbriae, and the tunica muscularis of the ampulla is thin. The muscular layer of the isthmus, however, is very well developed. A sphincter of circular smooth muscle is formed at the uterotubal junction.[1,4]

The mucosa of the infundibulum is highly folded longitudinally and covered by columnar epithelium, which is frequently ciliated. Changes in the estrous cycle are reflected by alterations in ciliogenesis and ciliary action. The mucosa of the ampulla is extremely plicated with primary, secondary, and even tertiary folds. This complexity of folding is more obvious in the mare than in ruminants, but the mucosa of the isthmus has much less folding than that of ampulla.[1,4,7]

The oocyte with its cumulus of granulosa cells is swept into the infundibulum by cilia. Very little, if any, follicular fluid enters the uterine tube following ovulation. The proximal portions of the oviducts provide a conduit for transport of the oocyte to the site of fertilization at the

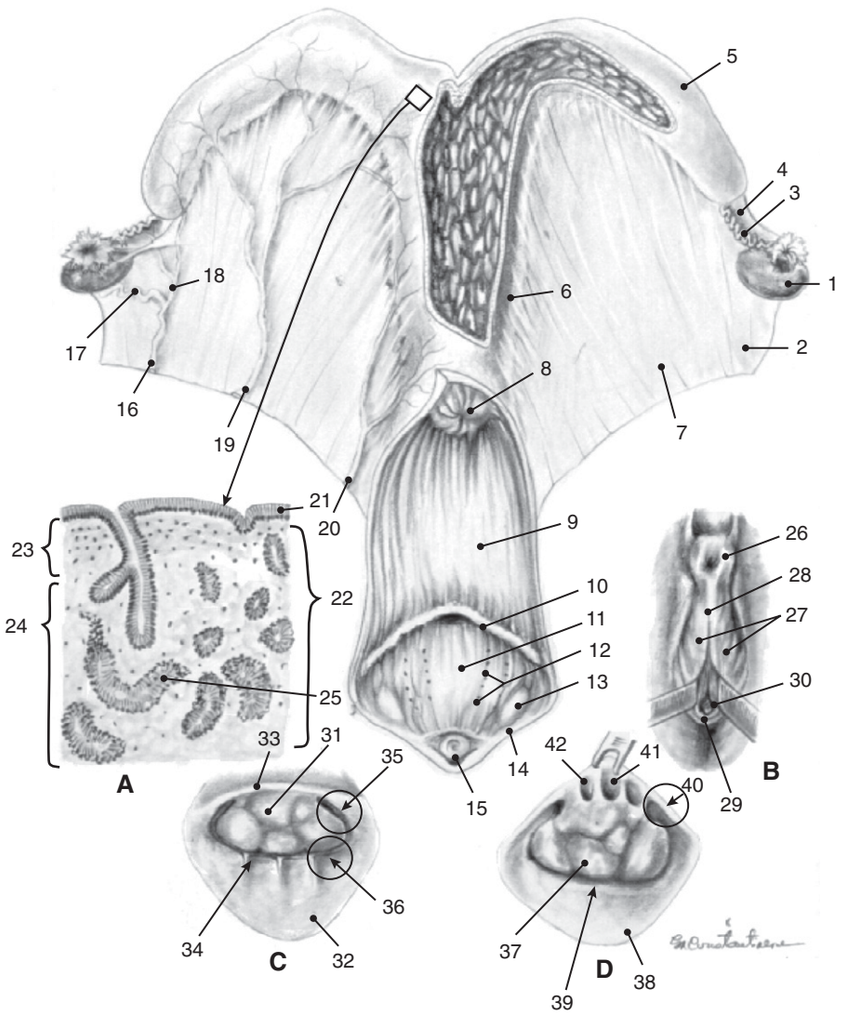

Fig. 7-5 Genital apparatus of the mare (dorsal view); the dorsal wall of the right horn and the body of the uterus, vagina, vestibule, and vulva are removed. *Inset A,* High-power magnification of a section of the endometrium. *Inset B,* The vulva. *Inset C,* The glans clitoridis. *Inset D,* The fossa and sinuses of the clitoris. 1, Ovary; 2, mesovarium; 3, uterine tube; 4, mesosalpinx; 5, uterine horn; 6, uterine body; 7, mesometrium; 8, vaginal portion of uterine cervix; 9, vagina; 10, transverse fold (hymen); 11, vestibule; 12, minor vestibular glands; 13, vestibular bulb; 14, vulvar labium; 15, glans clitoridis; 16, ovarian a.; (17, ovarian branch; 18, uterine branch); 19, uterine a.; 20, uterine branch of vaginal a. *Inset A:* 21, luminal epithelium; 22, lamina propria; (23, stratum compactum; 24, stratum spongiosum); 25, endometrial glands. *Inset B:* 26, anus; 27, vulvar labia; 28, dorsal commissure of vulva; 29, ventral commissure of vulva; 30, glans clitoridis. *Inset C:* 31, glans clitoridis; 32, ventral commissure of the labia; 33, preputium clitoridis (transverse frenular fold); 34, fossa clitoridis; (35, lateral recess; 36, ventral recess). *Inset D:* 37, glans clitoridis; 38, ventral commissure of the labia; 39, fossa clitoridis; (40, lateral recess); 41, medial sinus; 42, lateral sinus.

junction of the ampulla and the isthmus. The uterotubal junction allows spermatozoa to enter the isthmus following breeding, yet there is no reflux of uterine contaminants into the oviduct. The isthmus provides sites for capacitation and storage of spermatozoa while they await an oocyte to fertilize. The nonciliated epithelium of the uterine tube is predominantly secretory in nature and produces proteins and glycosaminoglycans. These secretory products may play a role in mediating capacitation of spermatozoa, fertilization of the ovum, and early embryonic cleavage. Preovulatory follicular fluid has a role in regulating oviductal protein production in vitro and in vivo (uterine tube ipsilateral to ovulation).[4] The mare is unique among domestic mammals in that only fertilized oocytes (developing embryos) enter the uterus approximately 6 days following ovulation. Unfertilized ova are retained in the middle third of the uterine tube for several estrous cycles among collagenous material that may contain cells similar to fibroblasts. Some have suggested this selective transport of fertilized oocytes is the initial step in "maternal recognition of pregnancy" in the mare.[1,4,5]

Clinical aspects. The uterine tube is the one portion of the reproductive tract of mares where functional abnormalities or pathologic changes and their significance are less likely to be appreciated. The myoelectrical activity of the myosalpinx can be influenced by oxytocin and prostaglandin $F_{2\alpha}$ (PGF). The clinical relevance of these modifications in oviductal function is yet to be determined. The presence of debris in the uterine tube has been described and is hypothesized to have an effect on tubal patency. Several techniques have been developed for the assessment of oviductal patency, but controversy exists over the prevalence of nonpatent oviducts and whether the observed debris actually represents a pathologic change.[4,22] Hydrosalpinx is rare in mares, but cases have been reported.[3,4,16,21] Mild to moderate inflammation and adhesions of the uterine tube have been noted in postmortem examinations of the horse; yet, as with other conditions observed in the uterine tubes, it is not known which oviductal changes have a negative effect on fertility and which represent normal variations. Several types of parovarian cystic structures occur in the vicinity of the oviducts. Some of these cysts are of mesonephric origin (epoöphoron and paroöphoron cysts). Although generally small and clinically significant, some of these structures, especially epoöphoron cysts, can become fairly large in the mare.[4,13,16,21] Despite being listed as mesonephric in origin by some references, hydatids of Morgagni are, based on histologic evidence, paramesonephric in origin and represent accessory uterine tubes, which can become quite large and impede proper function of the reproductive tract.[4,16,21]

The Uterus

The uterus is a dynamic organ, which has several diverse functions. The uterus facilitates spermatozoal transport to the uterine tubes, and optimal embryonic and fetal development depend on an appropriate uterine environment. Fetal transport from the maternal to external milieu at the expected time requires coordination of activity between the uterus, fetus, and various neuroendocrine organs. Elimination of contaminants following breeding and rapid postpartum uterine involution require efficient uterine defense mechanisms. The cervix serves as a barrier to foreign materials during pregnancy and allows passage of a foal at parturition. Changes in ovarian steroid production or alterations in uterofetoplacental hormone metabolism can cause uterine anatomy and function to vary.[1,4]

External structure and location. Embryologically derived from the paramesonephric ducts, the uterus of mares is bicornuate with a relatively long body. The uterus consists of paired uterine horns, a single body, and the cervix (see Figs. 7-1, 7-2, and 7-5). The cervix represents the thickened caudal portion of the uterus. The terminal segment of the uterine cervix (portio vaginalis) projects into the vaginal lumen. Externally, it is difficult to differentiate the uterine body from the cervix; however, the cervix can readily be distinguished by palpation. The surface of the uterus is covered by serosa or visceral peritoneum (perimetrium), which is continuous with the mesothelium in the broad ligaments. Viewed from above, the uterus is Y- or T-shaped. In nonpregnant mares, each uterine horn varies in length from 20 to 25 cm. In parous mares the horns may be asymmetrical. The uterine body is approximately 20 cm long. The cervix averages 5 to 7 cm in length and 3 to 4 cm in width. The intercornual ligament is much smaller and less distinct than in cattle. The uterus is capable of wide variations in size, shape, and location depending on the stage of the estrous cycle or pregnancy, seasonal influences, and degree of handling. The uterine horns are generally located entirely in the abdominal cavity. In the nonpregnant mare, the uterine body is found immediately in front of and frequently just ventral to the cranial brim of the pelvis. It can also be partially located in the pelvic cavity where it is continuous caudally with the cervix.[1,4–7,10,11]

Internal structure and function. As discussed previously, at the tip of each uterine horn, there is a small papilla containing a sphincter of circularly arranged smooth muscle fibers where the uterine ostium of the oviduct is located (Fig. 7-5). The dynamic nature and functional significance of the uterotubal junction have already been discussed. Where the uterine horns join the body, there is a short median septum. This septum and the papillae of the uterotubal junctions are important structures to be identified during endoscopic examination of the uterus (hysteroscopy) or nonsurgical deposition of semen within the oviduct.[1,4,12,13,22–24]

In contrast to ruminants, there are no caruncles (permanent elevations) in the mare's endometrium. The endometrial surface is composed of longitudinal folds that range in number from five to fifteen (Fig. 7-5). These endometrial folds in conjunction with the relatively flaccid uterine walls result in the existence of capillary spaces between adjacent folds rather than a central uterine lumen. It has been theorized that capillary action is partially responsible for the movement of secretions and inflammatory cells that collect in these spaces.[1,4,7,10] It seems logical that similar factors may contribute to rapid transport of spermatozoa from the cervix to the uterotubal junction.

The internal uterine ostium is funnel-shaped and is the beginning of the cervical canal. The external uterine ostium is located in the intravaginal segment of the cervix (portio vaginalis). Longitudinally folded like the endometrium of the uterine body and horns, the mucous membrane lining the cervical canal is pale rather than the typical reddish brown (ranges from yellow to reddish brown) observed in the rest of the uterus. The circular folds (plicae circulares or annular rings) present in the cervical canal of ruminants are absent in the cervix of the mare. Folds of mucous membrane extend through the external uterine ostium. The dorsal and ventral folds may continue onto the vaginal wall to form frenulums dorsally and ventrally, respectively (Fig. 7-6).[1,4,11–13]

The uterine wall is divided into the perimetrium, myometrium, and endometrium. The myometrium is composed of an outer layer of longitudinally oriented muscle fibers, a middle vascular layer, and a thick inner layer of circular smooth muscle. The vascular and longitudinal muscle layers are continuous with the vasculature and musculature, respectively, in the mesometrium. The endometrium (mucosa) consists of simple columnar epithelium overlying a lamina propria (Fig. 7-5, Inset A). The height of the cells and the degree of ciliation is dependent on the stage of the estrous cycle. The endometrial folds are formed by aglandular, longitudinally arranged cores of connective tissue that are covered by mucosa. The lamina propria of the endometrium extends from the basement membrane of the epithelium to the inner layer of myometrium. There is no muscular tissue in the lamina propria, which consists of the 1-mm thick stratum compactum (high density of stellate, stromal cells) and the stratum spongiosum (low density of cells with abundant interstitial fluid and prominent tubular branched glands).[4,25] These glands in the lamina propria are a distinguishing feature of the endometrium of the uterine body and horns. The glands open at the epithelial surface and extend to the depth of the lamina propria. Like the luminal epithelium, the lining of the endometrial glands consists of a single layer of columnar cells whose height is dependent on the stage of the estrous cycle and seasonal influences.[1,4,25,26] With regards to the pre- and postnatal development of these endometrial glands, it has recently been shown that the final differentiation and maturation of the secretory portion of the endometrial glands occurs after the first ovulation and requires exposure to progesterone following estrogen priming.[27] Gap junctions exist between adjacent cells in the luminal and glandular epithelium, and this feature of cells in the endometrium may allow for autocrine and paracrine functions of some hormones.[4] Endometrial glands have varying degrees of tortuosity and patterns of distribution depending on ovarian steroid hormone production and the time of the year. Straightened glands and endometrial edema are typically seen during the follicular phase of the estrous cycle (estrus).[1,25,26] During pregnancy, endometrial glandular secretions (histotrophs) and associated carrier proteins may play a role in establishing a normal uterine environment for embryonic and fetal development.[28] The diffuse epitheliochorial placentation (microcotyledonary) observed in the mare reflects the absence of caruncles and underscores the importance of the entire epithelium in supporting fetal maturation.[1,4,29]

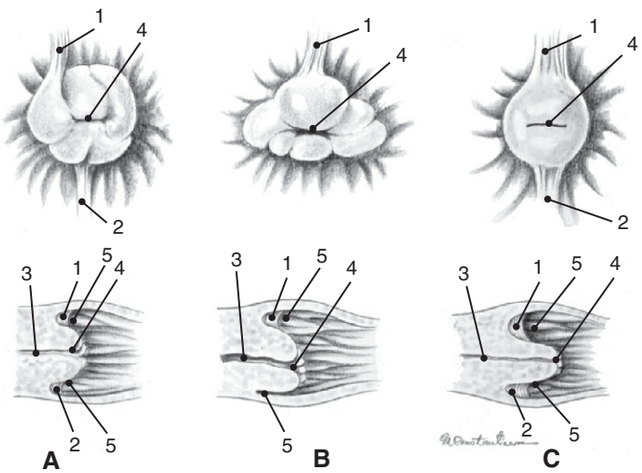

Fig. 7-6 *Upper row:* vaginal portion of the uterine cervix (caudal view). *Lower row:* median section through the vaginal portion of the uterine cervix. **A,** Diestrus. **B,** Estrus. **C,** Pregnancy. 1, Dorsal frenulum; 2, ventral frenulum; 3, cervical canal; 4, external uterine ostium; 5, vaginal fornix.

The endometrium plays an important role in the regulation of luteal function during the estrous cycle and pregnancy in the mare. The uterine mucosa is the source of the luteolysin PGF, and the embryo's ability to prevent endometrial synthesis and release of PGF and facilitate "maternal recognition of pregnancy" will be discussed later in this chapter.[1,4,29] In a process unique to the mare, involving metalloproteinases, chorionic girdle cells (fetal origin) initially attach to and then invade the luminal epithelium beginning at approximately day 35 of gestation. These trophoblastic cells engulf the epithelium, infiltrate the lamina propria, and differentiate into endometrial cups. Endometrial cups, which completely regress by the end of the fifth month of pregnancy due to a maternal cellular response, are specialized secretory structures that play a role in maintenance of the primary CL and the formation of supplementary CLs (also called secondary CLs by some authors) during gestation (second and third luteal response to pregnancy, respectively). The endometrial cups have a functional duration of 60 to 80 days that is independent of fetal viability. There may be a delay in return to normal ovarian and behavioral cyclicity following pregnancy termination if functional endometrial cups are present.[1,4,29]

Histologically, the structure of the uterine cervix reflects its function. There is a large number of mucigenous cells in the cervical epithelium. The cervix produces mucus, which serves as a lubricant or sealant depending on the stage of the estrous cycle or pregnancy. As compared to the uterine wall in the horns and body, there is an increased amount of collagen, as well as a prominent layer of circular smooth muscle containing many elastic fibers in the cervical region. The ability of the cervix to constrict and dilate is critical for successful breeding, pregnancy, and parturition.[1,4]

Clinical aspects. The ability to properly locate the uterus and to recognize the normal size, shape, and appearance of this organ, depending on the hormonal milieu and stage of pregnancy, is necessary to accurately diagnose uterine pathology and to make decisions regarding breeding and therapy. The uterus (including the cervix) is commonly examined using transrectal palpation or ultrasonography for assessment of the stage of the estrous cycle, pregnancy, postpartum uterine involution, and uterine disease. Additionally, examination of the cervix (portio vaginalis) by vaginoscopy provides useful information that complements the findings of palpation and ultrasonography (Figs. 7-6 and 7-7).[4,13,14]

Prior to the availability of diagnostic ultrasonography, uterine and cervical "tone" were the parameters most commonly evaluated to determine the stage of the estrous cycle. Unlike the cow, turgidity of the uterus and cervix increases between estrus and diestrus. During anestrus, the uterus is normally flaccid and nonedematous. The failure of some maiden mares' cervices to soften and dilate appreciably, even under the influence of estrogens, and the edema frequently associated with estrus can lead to inaccuracies in interpretation of palpation findings. Transrectal ultrasonic examination of the mare's uterus (including the cervix) allows visualization of the prominent endometrial folds ("cut surface of an orange" or "spokes of a wagon wheel" appearance), cervical edema, and increased diameters of the uterine horns, body, and cervix observed when the uterus is under the influence of estradiol during estrus (Fig. 7-6, *A*). Likewise, the absence of these features during ultrasonic imaging may suggest that the uterus is under the influence of progesterone secreted during the luteal phase of the cycle (Fig. 7-6, *B*).[1,4,13,14] Pregnancy diagnosis is performed routinely using transrectal palpation or ultrasonography.[1,4,29]

The rapidity of postpartum involution in the mare has been well documented. Mares can conceive and carry a foal to term when bred during the first estrus after parturition (foal heat). Transrectal ultrasonic imaging of the postpartum uterus has been successfully used to detect intraluminal fluid at the time of the foal heat. This accumulation of fluid indicates delayed uterine involution, which has an adverse effect on foal heat conception rates.[1,3,4] Various pathologic conditions can also be diagnosed using transrectal ultrasonic imaging of the uterus, including endometritis with small to moderate amounts of intraluminal fluid, mucometra, pyometra, and endometrial cysts (although there is some debate over the pathologic significance of these cystic structures).[1,3,4,13,14,30]

The mucosal folds of the portio vaginalis of the cervix can have a distinctive appearance during the various stages of the estrous cycle and pregnancy (Fig. 7-6). Their appearance may also change due to seasonal influences. Pink, moist, soft, edematous folds lying on the floor of the vagina with a prominent dorsal frenulum are typically seen during estrus, particularly in pluriparous mares. This "wilted rose" appearance is most apparent a day or two prior to ovulation, and it is at this time that the external uterine ostium appears as a horizontal slit (Fig. 7-6, *B*). During diestrus the folds have a "rose bud" appearance. The mucosa is pale and dry and the dorsal frenulum is less evident than during estrus. The terminal portion of the cervix projects further into the vagina and a ventral frenulum is sometimes observed, especially in maiden mares (Fig. 7-6, *A*). During early pregnancy the

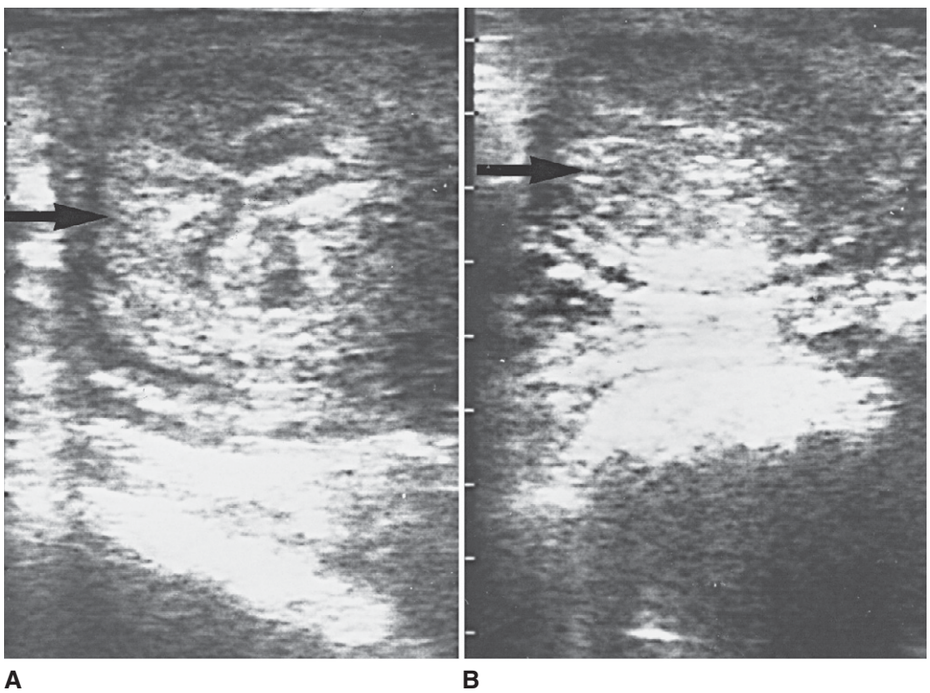

Fig. 7-7 Ultrasonographic appearance of the uterine horns during estrus demonstrating endometrial folds **(A)** and during diestrus **(B).** (Photographs courtesy of Mr. Howard Wilson.)

cervix appears "tight" and is often almost white in appearance. It projects even further into the cranial vagina than during diestrus, and the dorsal and ventral frenula are frequently both evident (Fig. 7-6, *C*). As pregnancy progresses, the cervix is pulled cranioventrad and is more difficult to view. Thick, "waxy" appearing mucus is present in the cervical canal and may be observed at the external uterine ostium and on the vaginal walls during pregnancy. During anestrus the cervical folds are nonedematous, pale, and soft. The cervix may be described as atonic, and it may be possible to observe the uterine lumen through the cervix.[1,4,7] Bruising of the mucosal folds at the external uterine ostium frequently occurs during coitus due to physical contact with the glans portion of the stallion's penis. Observation of bruises during vaginoscopy can suggest recent breeding in instances in which such information has inadvertently been omitted from the reproductive history.[4]

Endometritis, particularly of bacterial origin, is one of the most frequently diagnosed (correctly or incorrectly) abnormalities of the reproductive tract of the mare. It has been hypothesized that "breakdown" of uterine defense mechanisms, possibly related to trauma or abnormalities in anatomic conformation, predispose certain mares to endometritis. Humoral and cellular responses to uterine contaminants have been evaluated, and, most recently, considerable research has focused on the actual physical clearance of foreign material, including microorganisms, from the uterus (uterine clearance).[31] It has recently been proposed that an intrinsic defect in myometrial function predisposes individual mares to delayed uterine clearance.[32] The clinical diagnosis and treatment of endometritis and its underlying cause(s) involve a thorough examination of the mare's reproductive tract and require an understanding of normal reproductive anatomy and physiology. The reproductive tract of the mare may be examined utilizing ultrasonographic, vaginoscopic, histologic, cytologic, microbiologic, and, possibly, even hysteroscopic or scintigraphic techniques.[13,14,22,30,31]

Endometrial periglandular fibrosis is the hallmark of endometriosis, formerly termed chronic degenerative endometritis. The deposition of collagen around individual glands or glandular nests has been linked to recurrent endometritis, embryonic and fetal death, and abortion.[1,4,25] Recent studies have suggested that impaired synthesis or secretion of proteins by endometrial glands may be associated with endometriosis and may contribute to pregnancy wastage. It has also been demonstrated that specific enzyme systems and myofibroblastic transformation may play a role in the pathogenesis of endometrosis.[33–35] The detection of endometrial periglandular fibrosis is accomplished by evaluation of endometrial biopsy samples. Normal variations due to stage of the estrous cycle (edema during estrus or changes in luminal and glandular epithelial height) and seasonal influences (glandular atrophy or nonfibrotic clumping of glands during the anovulatory period) are taken into consideration. Various grading systems have been developed that demonstrate an inverse relationship between amounts of uterine fibrosis and the chances of an individual mare conceiving and carrying a live foal to term.[4,25,35,36]

A variety of other pathologic conditions may occur that require an awareness of normal uterine structure, function, and development. Uterine masses (hematomas and leiomyomas), transluminal adhesions, segmental aplasia, and double cervices (incomplete fusion of the paramesonephric ducts) have been reported in the literature.[21] It has been stated that freemartinism does not occur in the horse; however, asymptomatic XX/XY chimerism has been described in fillies born as twins to colts. Congenitally incompetent cervices and cervical lacerations, which can lead to subfertility when undiagnosed and not repaired, do occur and are diagnosed most accurately by digital and vaginoscopic examinations performed during diestrus.[4,13,14]

Ovarian and Uterine Vasculature

The important hormonal coordination of ovarian and uterine functions and the dynamic morphology of these organs underscores the significance of their blood supply. The CL is reported to be one of the most vascularized structures in the mare. The expansion noted in the uterine vasculature within the broad ligaments during gestation is unrivaled anywhere else in the body. The blood vessels of the ovaries and uterus have been described in detail in several articles and anatomy texts. The vasculature will be reviewed briefly in this chapter with an emphasis on clinically important structures and their functions.[1,4]

Nomenclature and Structure

The arterial blood supply and venous drainage for each ovary are provided by the ipsilateral ovarian branches of the ovarian arteries and veins, respectively, which are located in the mesovarium. Several small vessels form the ovarian branch of the ovarian vein, and these small veins anastomose with each other and with vessels of the uterine branch of the ovarian vein to form a uteroovarian plexus. The arterial supply to the mare's uterus comes principally from the uterine artery (formerly called the middle uterine artery), which arises on each side from the external iliac rather than from the internal iliac artery or one of its branches, as in other species. The uterine branches of the ovarian artery and the vaginal artery (previously referred to as the cranial and caudal uterine arteries, respectively) also contribute arterial blood to the uterus (see Figs. 7-1 and 7-5). The uterine branches of the ovarian and vaginal veins, as well a uterine vein, which is recognized by some authors, provide for uterine venous drainage. These vessels anastomose extensively with each other and with the corresponding structures on the other side of the body.[1,4,7–11]

Functional and Clinical Aspects

The intimate anatomic and functional relationship observed between ovarian arterial supply and uterine venous drainage in ruminants is absent in the mare. In cows and ewes, there is strictly a local (unilateral luteolytic; utero-ovarian) pathway with regard to the circulation of endometrial luteolysin. In the mare, endometrial PGF, secreted in the absence of an embryo or due to inflammation, enters the venous system, circulates

through the heart and lungs, and eventually reaches the ovaries via their arterial supply (systemic pathway).[1,4,29]

The structure, function, and location of uterine and ovarian blood vessels during gestation are relevant from a clinical perspective. Pre- or postpartum rupture of the external iliac, uterine, or ovarian arteries in the mare can lead to exsanguinations. The blood supply to the gravid uterus, the vascular layer of the myometrium, and the diffuse nature of equine placentation require a hemostatic suture the incised uterine wall during cesarean section to prevent excessive hemorrhage. Any conditions adversely affecting arterial blood supply or venous drainage of the ovaries or uterus during pregnancy pose a potential risk to the fetus.[1,4]

The Vagina

The vagina makes up a major portion of the anatomic connection between the uterus and the external environment. Although frequently only a slit due to dorsal compression by rectal contents, the vaginal lumen must be able to expand to accommodate the stallion's penis during breeding, the body of a foal during parturition, and a veterinarian's arm during diagnostic or therapeutic procedures.[1,4]

Location and Structure

For purposes of this discussion, the vagina of the mare represents the caudalmost derivative of the paramesonephric ducts and is sometimes called the vagina proper (see Figs. 7-2 and 7-5). The vestibule will be discussed separately later in this chapter. Located in the pelvic cavity, the vagina is approximately 20 to 25 cm in length and extends from the recess surrounding the terminal segment of the uterine cervix (vaginal fornix) to the prominent transverse fold overlying the external urethral orifice, which is a remnant of the hymen. Only the cranial portions of the vagina are covered by peritoneum. The extent of this coverage is determined by the variations in size of the rectogenital and vesicogenital pouches dorsally and ventrally, respectively. Collagenous connective tissue, fasciae, and muscles surround the portion of the vagina that is not covered by peritoneum.[1,4,6,7,10,11] Like the myometrium, the muscular layer of the vagina comprises a thinner longitudinal layer of smooth muscle and an inner, thicker layer of circular smooth muscle. The lamina propria of the vagina is well vascularized but is aglandular. Stratified squamous epithelium, consisting of basal, middle, and superficial regions, covers the vaginal mucosa, which is arranged in longitudinal folds.[1,4]

Functional and Clinical Aspects

The distensibility of the vaginal walls can be attributed to the muscular layer, the presence of fibroelastic tissue, and the longitudinal mucosal folds. Although variations in the mare's vaginal epithelium have been reported during the estrous cycle, the degree of cornification of superficial squamous cells is minimal compared to that observed during estrus in the bitch. As a result, vaginal cytologic testing is not useful for determination of the stage of the estrous cycle in mares. The vaginal canal can be used as a surgical approach to the peritoneal cavity (colpotomy) or may be a route for peritoneal contamination from coital or parturient trauma. Vaginal vessels, which are branches of the internal pudendal artery and vein, are located in the lateral walls of the vagina and should be taken into consideration when evaluating traumatic injuries or when performing surgical procedures in the vagina.[12] The transverse fold by virtue of its contribution to the vestibulovaginal sphincter is an important barrier to contamination of the more cranial aspects of the reproductive tract associated with pneumovagina or vesicovaginal reflux (urine pooling). The transverse fold is an important landmark to identify in order to avoid the passage of a vaginal speculum, insemination pipette, culturing device, or biopsy instrument into the urethra. Surgical modification of the transverse fold is involved in correction of urine pooling.[12] Persistent hymen occurs occasionally, and rupture during breeding may result in mild discomfort and minor hemorrhage. If imperforate, a persistent hymen can cause accumulation of fluid in the vagina and uterus. Like the uterus, the vagina is frequently bacteriologically sterile, but approximately 30% of mares have vaginal contamination with nonpathogenic bacteria. The vagina can be examined with a speculum or digitally for the presence of traumatic lesions, transluminal adhesions, persistent hymen, or urine pooling.[1,4,13,14]

The Vestibule and Vulva

Frequently described as part of the vulva in English and French textbooks, the vestibule is considered by the NAV to be a portion of the vagina. For purposes of this discussion, it will be considered distinct from the vulva but contiguous, having a common embryonic origin, similar functions, and shared structures. Because of its proximity and close relationship to the vestibule and vulva, the perineum (and related structures) will be discussed as well.[1,4,6,8,9,11]

Location and Structure

The vestibule and vulva represent the portions of the mare's reproductive tract caudal to the transverse fold of the vagina (see Figs. 7-1, 7-2 and 7-5). Originating embryologically from the urogenital sinus, these are the terminal and common segments of the reproductive and urinary tracts. The vestibule has a ventrodorsal slope with the ventral wall (floor) of the vestibule being longer than the dorsal wall, which is covered by the perineal septum. The floor of the vestibule is 10 to 12 cm long and extends caudally beyond the ischiatic arch. The vestibular walls and floor contain the constrictor vestibuli muscle, which consists of circular and longitudinal striated muscle fibers. Smooth muscle fibers, which can either be a continuation of the vaginal musculature or a part of the retractor clitoridis (formerly called the suspensory ligament of the anus), are also present. Medial to the constrictor vestibuli muscles are the vestibular bulbs, which are ovoid masses of erectile tissue measuring 7 cm by 3 cm (Fig. 7-5). The lamina propria of the vestibule is covered by stratified squamous epithelium. The mucosa of the vestibule is folded and is pink to reddish brown. Papillae on the vestibular floor and walls represent the

openings of the branched tubular minor vestibular glands.[1,4,6,7,10,11]

The vulva is just caudal to the vestibule and consists of two labia and the clitoris. The labia are apposed at the vulvar cleft and form a dorsal and ventral commissure (Fig. 7-5, Inset B). The ventral commissure is rounded and forms part of the prepuce of the glans clitoridis. Seventy percent of the vulva is normally below the floor of the pelvis. Each labium has a mucocutaneous junction between an internal, aglandular mucosa and a thin, external layer of skin. The skin is usually pigmented and is abundantly supplied with sweat and sebaceous glands. The constrictor vulvae are located beneath the vulvar skin and are composed of striated muscle fibers that are continuous dorsally with the sphincter ani (Figs. 7-1 and 7-2). Ventrally, the constrictor vulvae surround the clitoris, and a few muscle fibers cover the retractor clitoridis on each side.[1,4]

The clitoris of the mare is homologous to the stallion's penis and, like the penis, contains well-developed erectile tissue (corpus cavernosum clitoridis; Figs. 7-1, 7-2 and 7-5, Insets B, C, and D). The clitoris consists of a body (corpus clitoridis) consisting of two crura, which attach to the ischial arch and a prominent glans clitoridis. The corpus clitoridis lies beneath the vestibule and is approximately 5 cm long. The glans is normally 2.5 cm in diameter. The glans clitoridis is located in a small cavity, the fossa clitoridis, which is surrounded by the prepuce of the glans clitoridis, a portion of which is formed by the ventral commissure of the vulva. The transverse frenular fold, arising from the vestibular mucosa, forms the roof of the prepuce and is attached to the glans clitoridis by a median frenulum. There is a median sinus and, in most mares, two additional, but inconstant, lateral sinuses. These sinuses are located on the dorsal aspect of glans clitoridis (near the median frenulum). Within the fossa clitoridis are frenular, ventral clitoral and labial recesses. Smegma produced by clitoral sebaceous glands can be found in these areas (Fig. 7-5, Insets C and D).[1,4,6,7,10,11]

The portion of the body wall that surrounds the anal canal and vulvar and vestibular portions of the urogenital tract at the pelvic outlet is the perineum. The first few coccygeal vertebrae, the sacrosciatic ligaments, and the floor of the pelvis form the boundaries of the perineum dorsally, laterally, and ventrally, respectively. Embryologically, the perineum is formed by the connective tissue that separates the anal membrane dorsally from the urogenital membrane ventrally. This embryonic structure later develops into the perineal body, which is a node of muscular and fibrous tissue between the anus and the vestibule. The perineal region is distinct from the perineum and the perineal body and represents the cutaneous area between the semimembranosus muscles, extending from the base of the tail to the ventral commissure of the vulva (or base of the udder, according to some texts).[1,4,7,10,11]

Functional and Clinical Aspects

Owing to the upright posture of humans, there is a well-developed pelvic diaphragm consisting of muscle and a rudimentary tail, which supports the weight of the pelvic organs (and to a limited extent, the abdominal organs). The mare is a quadruped and the floor of the pelvis is the structure that bears the weight of the pelvic viscera. However, during urination, defecation, breeding, foaling, and episodes of tenesmus there are forces exerted upon the pelvic outlet that must be counterbalanced to prevent prolapse of the pelvic organs. In the mare, the levator ani and coccygeus muscles and connective tissue compose what is called the pelvic diaphragm (considered by some to be the muscular basis of the perineum). The constrictor vestibuli, constrictor vulvae, and retractor clitoridis, through their association with the vestibule and vulva, function with the pelvic diaphragm to provide for containment of the viscera within the pelvic cavity when expulsive forces are exerted (Figs. 7-1 and 7-2). As a result, uterine and vaginal prolapses are infrequent occurrences in the mare. The constrictor vestibuli, vestibular bulbs, and constrictor vulvae accommodate and secure the penis during breeding. The vestibular walls and labia of the vulva contain elastic fibers that allow for expansion during coitus and parturition. The constrictor vulvae function in the closure of the labia and the eversion of the clitoris ("winking") observed following urination or during estrus.[1,4,7–11]

The vulvar labia along with the transverse fold (as part of the vestibulovaginal sphincter) and the uterine cervix are the three anatomic barriers between the external environment and the uterus. Variations in perineal conformation can predispose mares to reproductive failure. Proper closure of the vulva is necessary to prevent air and feces from contaminating the reproductive tract. Generally, no more than 4 cm of the vulvar cleft should be between the dorsal commissure and the pelvic floor, and the vulvar angle (cranial-to-caudal slope of the vulva) should not vary more than 10 degrees from vertical (Figs. 7-1 and 7-2). Systems for quantifying abnormalities in perineal conformation have been devised, and surgical procedures have been developed to correct these variations.[4,12]

Bacteria, including some potentially pathogenic varieties, can often be cultured from the vestibule and fossa clitoridis of the mare. This bacterial flora has the potential to contaminate more cranial portions of the reproductive tract during the performance of diagnostic and therapeutic procedures. The various sinuses and recesses of the glans clitoridis and fossa clitoridis collect smegma (Fig. 7-5, Insets C and D). The medial sinus of the glans clitoridis may be 1 cm deep and provide adequate anaerobic conditions for growth of *Taylorella equigenitalis,* the bacteria that causes contagious equine metritis (CEM). Surgical removal of the glans and sinuses has been performed in countries where CEM is endemic.[1,4]

Congenital anomalies, traumatic injuries, and drug-induced anatomic abnormalities occur in the vestibular and vulvar region. Equine pseudohermaphrodites have been reported, and a penile structure, protruding from what appears to be labia in the perineal region, may be observed in affected horses. Perineal lacerations of various degrees may occur during foaling or breeding, and surgical procedures have been developed for their correction.[12] Hypertrophy of the glans clitoridis may be a clinical finding in mares to which anabolic steroids have been

administered. It is not uncommon for these mares to exhibit variable degrees of subfertility.[1,4,13]

The Epiphysis (Pineal Gland) and Hypothalamus

In humans and animals alike, the neuroendocrine functions of the pineal gland (epiphysis) and hypothalamus (inclusive of the hypophysis, or pituitary gland in this discussion) play an important role in regulating the body's physiologic processes. These structures facilitate development of the reproductive tract and endocrine regulation of the mare's estrous cycle. Sexual behavior, gestation, parturition, and lactation are all affected by the pineal gland and hypothalamus. Clinicians should be aware of the location of these structures and their interaction with one another and the impact their functions might have on reproductive physiology.

Location, Structure, and Function

The pineal gland is pine-cone shaped in humans (hence the name pineal). In the horse, the pineal gland is an ovoid, reddish brown structure 10 to 12 mm long and 7 mm wide and is a component of the epithalamic region of the diencephalon (Fig. 7-8). It is located caudodorsal to the thalamus, protected in a deep median depression between the cerebral hemispheres and the vermis of cerebellum. The pineal gland is surrounded by a capsule of pia mater from which trabeculae project into the gland, dividing it into masses of epithelial cells of ependymal origin. These cells secrete melatonin, which plays a role in the regulation of reproductive photoperiodicity and possibly puberty. The rete pinealis supplies arterial blood to the pineal gland, and venous drainage is provided by the ventral longitudinal sinuses.[1,4,7,10]

The hypothalamus comprises the portion of the diencephalon that includes the ventral walls of the third ventricle, the mammillary body, the tuber cinereum, and the hypophysis. Cell bodies of neurosecretory cells are organized into discrete foci (nuclei).[1] Magnocellular (large) neurosecretory cells such as those found in the supraoptic and paraventricular nuclei secrete hormones such as vasopressin and oxytocin at their terminal nerve endings in the neurohypophysis.[1,4,7,10] Releasing and inhibitory (hypophysiotropic) factors such as gonadotropin-releasing hormone (GnRH), thyrotropin releasing hormone (TRH), and dopamine (prolactin inhibitory factor; PIF) are synthesized and secreted by predominantly parvicellular (small) neurosecretory cells located in a variety of hypothalamic nuclei. These hormones are carried to the appropriate target cells in the adenohypophysis by the hypothalamic-hypophysial portal system (to be discussed later).[1,4,7]

Although a part of the hypothalamus, the pituitary gland warrants special attention because of its unique origin, structure, and functions. The hypophysis is flattened dorsoventrally and is approximately 2 cm wide. It comprises the neurohypophysis (light-colored "posterior pituitary gland"), which is of neuroectodermal origin, and the adenohypophysis (dark-colored "anterior pituitary gland"), derived from an evagination of oral ectoderm (Rathke's pouch; Fig. 7-8).[1,4,7] The major portion of the gland is covered by dura mater in the hypophysial fossa of the basisphenoid bone. The hypophysis is attached to the rest of the hypothalamus by the infundibular (neural stalk) portion of the neurohypophysis, which is a continuation of the tuber cinereum at the median eminence. The adenohypophysis can be subdivided into the pars distalis (analogous the human "anterior pituitary gland"), pars intermedia, and the pars tuberalis, and, together, these structures almost surround the entire neurohypophysis. The pars distalis contains specific cell types which, with appropriate stimulation, secrete prolactin, the gonadotropins (follicle-stimulating hormone or FSH and luteinizing hormone or LH), thyrotropin (thyroid-stimulating hormone or TSH), or other endocrine products. Cells in the pars intermedia secrete melanocyte-stimulating hormone in some species, and the pars tuberalis contains numerous vessels of the hypothalamic-hypophysial portal system and some secretory cells, such as those which secrete the gonadotropins, which are also referred to as gonadotropes.[1,4,7,10,11,37] The

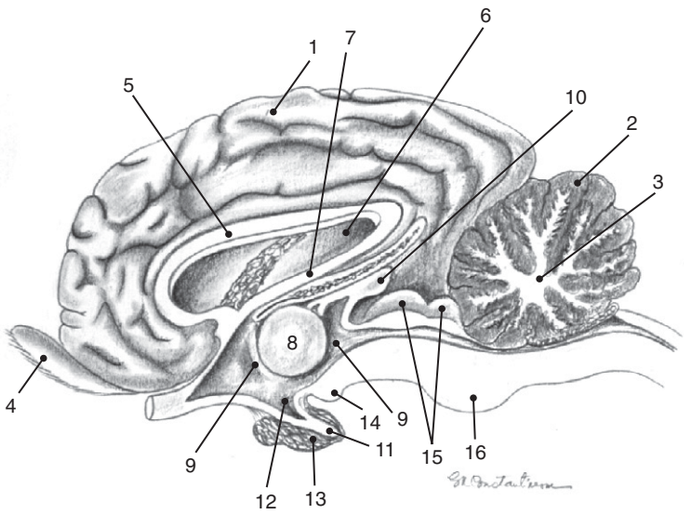

Fig. 7-8 Median section thorough the brain. 1, Cerebrum; 2, cerebellum; 3, vermis; 4, olfactory bulb; 5, corpus callosum; 6, hippocampus; 7, fornix; 8, interthalamic adhesion; 9, third ventricle; 10, epiphysis (pineal gland); 11, neurohypophysis; 12, infundibulum; 13, adenohypophysis with pars tuberalis (covering the infundibulum, pars distalis, and pars intermedia); 14, mammillary body; 15, corpora quadrigemina; 16, pons.

proportion of gonadotropes in the pars tuberalis, unlike that in the pars distalis, appears to be increased in normally cycling, sexually active mares, as compared to seasonally anestrous mares. In addition, although gonadotropes in the equine adenohypophysis frequently produce both FSH and LH, cells that specifically secrete FSH or LH have been shown to differentiate predominantly in the adenohypophyses of cycling mares.[37]

The hypothalamic vasculature contributes to the functional relationships between the hypophysis and the other portions of the hypothalamus. Branches of the internal carotid artery and the rami communicantes caudales supply the capillary plexus in the median eminence. Portal vessels in the pars tuberalis, which terminate in sinusoidal capillaries, transport endocrine products (releasing and inhibitory factors) from the more dorsal parts of the hypothalamus to the adenohypophysis, and may, in fact, represent the sole arterial supply to this part of the pituitary gland. The caudal infundibular arteries supply arterial blood to the neurohypophysis. Venous drainage for the hypothalamus is provided by the cavernous and intercavernous sinuses.[1,4]

Clinical Aspects

Manipulation of the photoperiod to inhibit melatonin production by the pineal gland is commonly utilized in reproductive management of broodmares to facilitate the commencement of normal estrous cycles earlier during the year. Based on research performed in rodent species and validated to some extent in the horse, this neuroendocrine pathway is somewhat complex and appears to be dependent on normal retinal function and the integrities of the optic chiasma, the retinohypothalamic nerve tracts and the suprachiasmatic and paraventricular nuclei. Projections from the median forebrain bundle and pre-and postganglionic sympathetic fibers associated with the superior cervical ganglion must also be intact for the effects of photoperiod on melatonin synthesis and mare reproductive function to be manifested.[38]

What has been traditionally referred to as the hypothalamic-pituitary-gonadal axis during discussions of reproductive endocrinology forms the basis for regulation of the mare's sexual development and estrous cycle. Pituitary adenomas and pars intermedia dysfunction may adversely affect reproductive function in the mare.[1,4] With regards to "fescue toxicosis," ergot alkaloids produced by the endophyte *Neotyphodium coenophialum* (formerly *Acremonium coenophialum*) interact with D_2-dopamine adenohypophysial receptors on maternal lactotropes and possibly fetal corticotropes to mimic hypothalamic suppression of prolactin secretion and delay parturition. Recently, it has also been noted that ergot alkaloid exposure may delay the onset of the ovulatory season and cause irregularities in the seasonal estrous cyclic activity of mares.[39–42]

The Mammary Glands

Adequate secretion of milk (including colostrum) must take place at the appropriate time for normal growth and development of the foal.

Location and Structure

Between the hind legs, closely attached to the ventral abdominal wall, is the mare's udder (Figs. 7-1 and 7-2). In maiden mares it is barely visible and is frequently situated caudal to two folds of abdominal skin, which are effaced during mammary development toward the end of gestation. In maiden mares, the teats are small and flattened laterally, and hair may be present at the orifices on the tips of the teats of young fillies. Removable, dark sebum is present in the intermammary groove between the teats. The halves of the udder (mammae) are supported by lateral and medial suspensory ligaments on each side. The mare has two teats, which generally have two or occasionally three orifices. For each teat orifice, there is a corresponding glandular complex, the largest of which is located cranially. Associated with each orifice is a separate papillary duct (streak canal) lined by stratified squamous epithelium (Fig. 7-2). The papillary duct is continuous with a lactiferous sinus, consisting of a papillary part (teat cistern) and a glandular part (gland cistern). Each lactiferous sinus, lined with bistratified columnar epithelium, leads into a lactiferous duct system and contiguous glandular tissue, lined with columnar epithelium. In addition to glandular tissue and a system of ducts, the udder contains smooth muscle, fibroelastic tissue, and well-developed vasculature. The cranial and caudal mammary arteries, which are branches of the external pudendal artery, supply arterial blood to the udder. Similarly named veins provide for venous drainage.[4]

Functional and Clinical Aspects

Mares can develop mastitis, and the severity of inflammation, the response to therapy, and the proportion of the mammary glands involved will determine the prognosis for future lactations. Galactorrhea in mares is not an infrequent complaint by clients and can have a number of causes, including pituitary adenomas. Ergot alkaloid exposure, such as in cases of "fescue toxicosis" or ergotism, suppresses the typical, periparturient secretion of prolactin resulting in inhibited lactogenesis and agalactia.[4,39–42]

REPRODUCTIVE PHYSIOLOGY

As there is an intimate relationship between organ structure and function, some important physiologic aspects of equine reproduction have already been discussed in conjunction with reproductive anatomy. The purpose of this section is to explain concisely what is presently understood about photoperiod regulation, the estrous cycle, puberty, pregnancy (including maternal recognition of pregnancy), and parturition in the mare. For a detailed description of the classification and description of the hormones involved in equine reproduction, the reader is directed to the corresponding chapter in the previous edition of this text.[4]

Photoperiod Regulation of Reproduction in Mares

It has long been recognized that reproductive function in the mare, specifically normal ovarian cyclic activity, is

governed predominantly by photoperiod, or, in other words, day length. This topic has been covered extensively in other references, but will be discussed briefly in this chapter in order to provide the physiologic basis for some clinically relevant aspects of mare reproductive management. In addition, other endocrine pathways and external factors traditionally considered to have minimal roles in the seasonal onset of estrous behavior and the regulation of equine reproductive function in the mare have recently been shown to play a larger part in these processes.[1,4,43–49]

To varying degrees, mares naturally respond to changes in day length and generally experience autumnal transition, winter anestrus, and vernal transition (collectively termed the anovulatory season), and seasonal polyestrous cyclicity (breeding or ovulatory season) during the late spring and summer. Photoperiod influences ovarian activity, at least in part, through the actions of melatonin. Melatonin is synthesized and secreted in the pineal gland mostly at night and is thought to act by decreasing the synthesis of GnRH in the hypothalamus. Decreasing day length in the fall results in lower hypothalamic GnRH content and reduced secretion of LH and FSH by the adenohypophysis. Clinically, mares may have reduced fertility and persistent, anovulatory follicles and, possibly, prolonged periods of estrus during the autumnal transition (receding phase). During the winter, hypothalamic GnRH is at its lowest concentrations, consistent with short photoperiods and a high level of pinealocyte melatonin synthesis.[1,38] Owing to the absence of GnRH activation of the promoter for the LH β subunit, the adenohyphysial content of LH is minimal during anestrus.[38,43] The concurrent decreases in ovarian activity and size are accompanied by passive sexual behavior in anestrous mares. Increasing photoperiod after the winter solstice (approximately December 22) results in a gradual decrease in melatonin synthesis and an increase in pulsatile secretion of GnRH. FSH secretion is subsequently stimulated and folliculogenesis is initiated. The relative lack of LH secretion is evidenced by the development of multiple, transitional, anovulatory follicles. The ovaries of mares in the vernal transition (resurging phase) are frequently described as resembling "clusters of grapes" by transrectal palpation, and ultrasonographically these ovaries contain multiple nonechogenic follicular structures.[1,15,16,38] Sufficient GnRH stimulation induces LH synthesis and secretion and ovulation. Subsequent to this initial ovulation in a previously anovulatory mare, the sequential and cyclic endocrine and functional reproductive events which characterize the equine breeding season take place.[1,38]

Several photoperiod manipulation techniques have been employed to hasten the onset of ovulatory estrous cycles in mares. Exposing mares to 16 hours of artificial light (10 to 12 foot-candles of light), or increasing day length after sunset for 2 to 3 hours for a total photoperiod of 16 hours have been used successfully to manipulate photoperiod. The introduction of an hour of light 9.5 hours after the onset of darkness has been suggested as another alternative to stimulate mares to cycle earlier in the year. Even with artificial photostimulation, mares will experience a "transitional" phase. Photoperiod manipulation schemes should commence at least 60 days before the desired time of breeding.[1,38]

Recent research has suggested that factors other than photoperiod and endocrine mediators in addition to melatonin may play roles in the commencement of normal cyclic behavior in mares. The tendency of some mares to cycle continuously without respect to photoperiod may reflect the influence of some of these external factors and other hormonal signals. Young mares have been shown to begin anestrus earlier than mature mares, and body weight, percent body fat, and circulating levels of leptin (reflecting energy availability) modify the photoperiod response and determine the propensity of individual animals to cycle continuously throughout the year.[44,45] Endogenous opioid tone is less during the breeding season than during anestrus, and mares cycling throughout the year do not experience such fluctuations.[46] Prolactin has been proposed to play a role in the regulation of seasonal cyclicity, particularly the vernal transition, and melatonin, dopamine, and opioids have been shown to decrease circulating levels of prolactin in the spring.[44,47–49] Ergot alkaloid–exposed, transitional mares have delayed ovulations in association with decreased circulating levels of prolactin.[40,41,47] Lower ambient temperatures appear to adversely affect the ability of D_2-dopamine receptor antagonists to hasten ovulation in anestrous and transitional mares.[40,47,48,50]

A variety of pharmacologic approaches have been developed to advance the onset of the ovulatory season in transitional mares. Exogenous progestins (with or without estradiol) and GnRH-type compounds have been successful to variable degrees in hastening ovulation. Human chorionic gonadotropin (hCG), by virtue of its LH-like activity, has been employed in some transitional mares with persistent follicles.[51] Stimulation of prolactin secretion by lactotropes, through the use of D_2-dopamine receptor antagonists, has also been recommended for use in mares experiencing the vernal transition.[40,41,47,48,50]

The Estrous Cycle

The normal estrous cycle of the mare is approximately 21 days, and the normal lengths of estrus and the interestrous interval range from 5 to 7 days and from 14 to 16 days, respectively. The estrous cycle of nonpregnant mares is characterized by a typical pattern of hormone concentrations in the peripheral blood (Fig. 7-9). Individual variations in estrous cycles exist and are dependent on seasonal influences, body condition, exposure to ergot alkaloids in tall fescue pastures or hay, the number of follicular waves, and the possibility of multiple or diestrous ovulation(s). An understanding of the normal equine estrous cycle and an awareness of the common observed variations are necessary for the efficient reproductive management of breeding programs. This knowledge allows for the identification of mares with irregular reproductive cycles and behavior, which require further examination and therapy. Based on the normal reproductive physiology of the mare, pharmacologic protocols have been developed for the induction and synchronization of estrus and ovulation.[1,4,51]

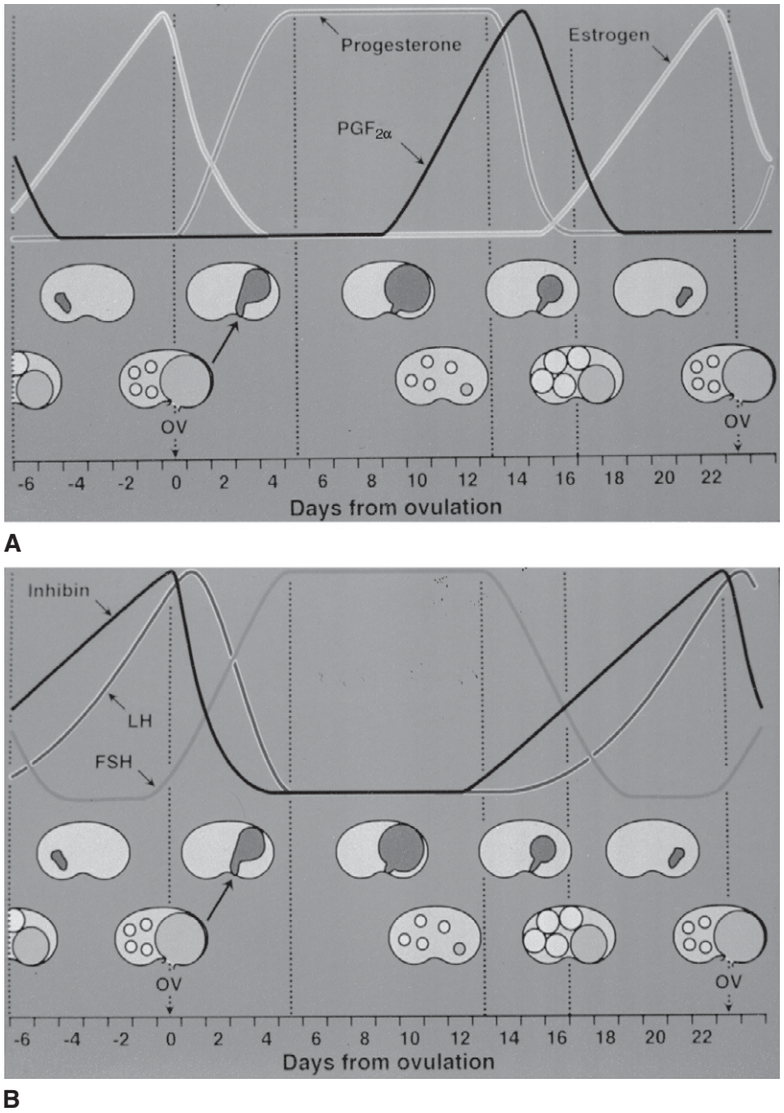

Fig. 7-9 Temporal relationships between circulating hormones and ovarian events. **A,** Progesterone, estrogen, and $PGF_{2\alpha}$. **B,** FSH, LH, and inhibin. (From Ginther OJ: *Reproductive biology of the mare: Basic and applied aspects,* 2nd ed. Cross Plains, WI: Equiservices, 1992. Reprinted with permission of O.J. Ginther. Photographs courtesy of Mr. Don Connor.)

Estradiol of follicular origin is the predominant reproductive steroid in the blood at the beginning of estrus. Progesterone is low at this time (less than 1 ng/ml), and mares should begin to show behavioral signs of estrus when teased (eversion of the clitoris, frequent urination, standing for the stallion, etc.). "Mouth clapping," along a head held low with ears turned back, are consistent signs of estrus in jennies and zebras. The dominant follicle grows in size and produces more estradiol. Subordinate follicles become atretic owing to production of inhibin (induced by estradiol) by granulosa cells of the dominant follicle. Under the influence of estradiol, GnRH pulse frequency increases (after an initial suppression) to favor secretion of LH relative to FSH. LH levels increase, and FSH reaches minimum levels. The preovulatory LH surge is more gradual in the horse, as compared to other species, and actually reaches maximum peripheral concentrations 1 to 2 days following ovulation. As LH levels increase and the follicle approaches ovulation, theca cells begin to degenerate, and estradiol production actually begins to decrease prior to ovulation. Blood levels of follicular estradiol and inhibin return to their basal levels over a 1- to 2-day period following ovulation, and this decline in estradiol coincides with the end of behavioral estrus in the mare (Fig. 7-9).[1,4] hCG and GnRH analogues have been used pharmacologically to induce ovulations in mares.[51]

A common clinical complaint in mares is abnormal prolongation or increased frequency of estrus. The athletic performance of some mares can be adversely affected by estrous behavior, and various therapeutic approaches to this problem have been employed.[52] As mentioned earlier, mares can return to estrus sooner than anticipated (short interestrous interval) because of endometritis and endotoxemia. The large quantities of estrogens synthesized during pregnancy may also result in mares exhibit-

ing prolonged estrous behavior during gestation. Mares with granulosa cell tumors may exhibit signs of persistent estrus, and older mares or mares during vernal transition may fail to ovulate.[1,4,14]

Multiple (predominantly double) ovulations occur in mares, and there seems to be an individual predilection for this phenomenon. There may also be a hereditary propensity for double ovulations as this is a fairly common occurrence in Thoroughbred and draft breed mares (approximately 20–25%) as compared to other breeds (5–10%). Maiden and barren mares are more inclined than foaling mares to have double ovulations, and it is postulated that double ovulations occur because of an increased number of LH receptors on two developing follicles (increased sensitivity to gonadotropins). Double ovulations can result in twin pregnancies, which, if maintained beyond the embryonic period (40 days), are frequently aborted.[1,3,4]

Diestrus begins following ovulation with cessation of estrous behavior. The predominant reproductive hormone during this period is progesterone. During diestrus, mares are generally disinterested in stallions and may pin their ears and kick when teased by stallions. Blood levels of progesterone increase rapidly following ovulation, reaching a maximum (greater than 4 ng/ml) 4 to 7 days after ovulation. Under the influence of progesterone, GnRH pulses are infrequent and FSH pulse amplitude increases relative to LH. Peripheral blood levels of FSH reach a maximum during diestrus, allowing for follicular development. Suppressions of subsequent levels of FSH and LH, as well as prolongation of the interovulatory interval, have been observed with pharmacologic doses of a GnRH analogue (deslorelin) implanted in mares to induce ovulation.[53,54] Recent research has suggested this undesired effect of deslorelin can be avoided with removal of the implant 48 hours after initial implantion.[55]

One (Fig. 7-9) or two waves of follicular growth (primary and secondary) may occur during diestrus. If there is one follicular wave, then this constitutes the primary wave of folliculogenesis. This follicular wave begins approximately 9 days following ovulation, and the granulosa cells of one follicle begin to produce inhibin, which causes a decline in FSH. The theca and granulosa cells of this dominant follicle begin to synthesize small amounts of estradiol, and the subordinate follicles begin to undergo atresia. At about the same time as the primary wave of folliculogenesis is occurring, the progesterone-primed endometrium begins to synthesize and secrete PGF.[1,4] It has been shown in pony mares that oxytocin secretion and endometrial oxytocin receptor density are correlated with endometrial synthesis and secretion of PGF.[56] Peak peripheral blood levels of PGF occur on or about day 14 or 15 following ovulation and coincide with luteolysis and a decline in blood levels of progesterone. Increased secretion of estradiol by the dominant follicle results in the mare's return to estrus 1 to 3 days following CL regression and the beginning of another estrous cycle (Fig. 7-9).[1,4]

Prolonged luteal activity (longer than normal diestrus) is commonly observed in mares, and there are several explanations for this condition in addition to the prolonged interovulatory period associated with deslorelin implants.[53,54] Late diestrous ovulations from secondary follicular waves (also called secondary ovulations) may result in a developing secondary CL (insensitive to PGF) being present at the time of normal PGF secretion by the endometrium, and severe uterine disease may render the endometrium incapable of PGF synthesis and secretion. Exposure of nonpregnant mares to ergot alkaloids in tall fescue pastures or hay has also been associated with prolonged luteal activity. Early embryonic loss after the normal time of maternal recognition of pregnancy (14–16 days) will also result in a longer than expected interestrous interval. Idiopathic persistence of the CL (in the absence of uterine pathology or embryonic loss) has been described, but it is felt by some that further objective documentation of this condition is warranted.[1,4,40] PGF or related compounds can be administered therapeutically to mares in cases of luteal phase prolongation or in order to shorten the interovulatory period for reproductive management purposes.[51]

PUBERTY

The onset of puberty in the equine female is denoted by occurrence of the first ovulation. It is generally thought to occur sometime between 12 and 24 months of age. The prepuberal ovary must undergo changes in morphology prior to ovulation, and these may occur as early as 5 to 7 months of age in the mare. Little research has been done on puberty in the mare because horse owners are generally content to allow mares to develop until the age of 3 prior to breeding. Additionally, some horses are initially trained and shown as yearlings and 2-year-olds and sexual immaturity under these circumstances may be desirable. In prepuberal animals, there is adequate FSH secretion to stimulate the development of small follicles, which secrete some estradiol but are not yet ovulatory. It has been speculated that the endocrine events and alterations in hormonal gene expression that signal the onset of puberty in horses share significant similarities with the factors that regulate the vernal transition and the onset of seasonal ovarian cyclic activity in mature mares.[1,4,38]

There appear to be several external determinants of the timing of the first ovulation in young mares. Prepuberal mares (6 to 8 months of age) exposed to 16 hours of light beginning in December actually ovulated later than control animals. Poor nutrition may delay the onset of puberty by its effect on body fat composition and growth rate. In cases of poor nutrition, it has been demonstrated that LH pulse frequency and amplitude are suppressed when growth rate is retarded.[1,4,44] Based on data collected for mature cycling mares, exposure to *Neotyphodium*-infected fescue hay or pastures, or potentially, other sources of ergot alkaloids might also be expected to delay the first ovulation in young mares.[40,41] Pheromones and exposure to male animals may play a role in puberty in the mare, as it does in other species such as the ewe. Anabolic steroids have been implicated as having a role in delaying normal reproductive cyclicity in young mares, and young mares with chromosomal abnormalities may fail to cycle.[1,4,14]

Pregnancy

The ultimate goal of breeding a mare is the delivery of a live foal. The normal estrous cycle of the mare must be interrupted through embryonic-maternal interactions, and adequate placentation must be established for the metabolic and endocrine support of pregnancy. The length of gestation varies in mares, ranging from 310 to 380 days (average 335–340 days), with male fetuses tending to have longer gestations. Pregnancy begins with conception (fertilization), which takes place within the oviduct of the mare. Approximately 24 hours after fertilization, the first cleavage takes place. On the sixth day after ovulation, the morula stage of the embryo is selectively transported in preference to nonfertilized ova from the isthmus of the uterine tube via the uterotubal junction to the ipsilateral uterine horn. This process is thought by some to represent a form of "maternal recognition of pregnancy" in the mare.[1,4]

Blood concentrations of progesterone (luteal origin) reach their peak by the time the embryo enters the uterus. Under the influence of progesterone, the uterus is prepared to provide the proper environment for early embryonic development. Concentrations of immunoassayable progestins greater than 2.5 ng/ml 12 days after ovulation have been reported to be associated with higher pregnancy rates in mares.[57] Embryonic blastocyst formation occurs with synthesis of the embryonic capsule occurring prior to shedding of the zona pellucida. Other dramatic, metabolic changes take place within the developing embryo, such as the synthesis and secretion of insulin-like growth factor–binding proteins, which facilitate the actions of insulin-like growth factors on the equine conceptus.[58] By virtue of the embryonic capsules' mucin-like glycoprotein structure, the secretion of estrogens by the embryo and maternal myometrial contractions, the horse embryo exhibits mobility within the uterus beginning on day 9 or 10 after ovulation. Coinciding with maximum embryonic mobility (days 11–15 following ovulation) diminished levels of PGF metabolites are observed in pregnant versus nonpregnant mares. It is theorized that the mobile horse embryo produces some anti-luteolysin, which prevents regression of the mare's CL, and this luteostatic mechanism has been termed the first luteal response to pregnancy ("maternal recognition of pregnancy"). The mechanism of this anti-luteolysin's action may involve stimulation of an inhibitor of endometrial PGF synthesis or interference with the oxytocin-PGF interaction within the endometrium.[1,4,56] Because equine embryos do not seem to express the appropriate genes, the anti-luteolysin in the mare is most likely not an interferon, as in the cow and ewe.[1,4]

Equine embryos fix (day 16 after ovulation) and continue in their development following the first luteal response to pregnancy. Blood levels of progesterone in the mare decline slightly from this time (decreasing size of CL) until the secretion of eCG by the endometrial cups on approximately day 40 of pregnancy. Resurgence of progesterone synthesis by the primary CL (resulting from eCG stimulation) has been termed the second luteal response to pregnancy. The formation of supplementary CLs, induced by eCG secretion, constitutes what has been referred to as the third luteal response to pregnancy. Blood levels of progesterone (secreted by primary and supplementary CLs) reach their maximum between days 60 and 100 of gestation (10–20 ng/ml) and plateau until days 120 to 150. By the end of the sixth month of gestation, progesterone levels reach a minimum (less than 2 ng/ml).[1,4]

Lower than expected progesterone levels may occur in the pregnant mare under several different circumstances. Ultradian rhythms of luteal progesterone secretion may be reflected in the timing of sampling. ELISA tests for progesterone will detect very little progesterone after days 120 to 150 of gestation. Endotoxin has been shown to cause PGF production and luteolysis, which can result in pregnancy loss prior to day 70 of pregnancy. Luteal insufficiency has long been thought to be a cause of embryonic and fetal death, although its actual incidence is subject to debate.[1,4]

Production of 5-α pregnanes by the uterofetoplacental unit begins around day 40 of pregnancy and reaches a plateau by approximately mid-gestation. Although the first edition of this text referred to these compounds collectively as "progestins," the fact that 5-α pregnanes include metabolites of pregnenolone, as well as progesterone, makes "progestagens" a more appropriate collective term for these compounds, including instances in late pregnancy where they are being measured by radioimmunoassays for progesterone.[4,57,59] The 5-α pregnanes may be able to maintain pregnancy in the absence of ovarian progesterone (as demonstrated in ovariectomy and endotoxin studies) as early as day 50 in some mares and frequently by day 70 of gestation.[1,4] Between days 90 and 300 of gestation, maternal plasma concentrations of immunoassayable progestagens are reported to range between 4 and 10 ng/ml in healthy pregnancies.[57] Thirty days prior to parturition, there is a dramatic rise in levels of progestagens in the maternal circulation, which peaks (usually at over 25 ng/ml for immunoassayable progestagens) 2 to 3 days prepartum and declines precipitously within 24 hours of parturition.[57,59]

Concentrations of progestagens in the maternal circulation are affected by a number of pathologic conditions. In cases of "fescue toxicosis" or other prolonged exposures of mares to ergot alkaloids, the dramatic increase in maternal immunoassayable progestagens observed during the last 30 days of pregnancy is suppressed or absent.[39–42,57,59] Withdrawal from *Neotyphodium coenophialum*–infected pastures or ergotized grains or grasses (30 days prepartum) or prepartum treatment with D_2-dopamine receptor antagonists prevents this "fescue toxicosis"–associated decrease in maternal immunoassayable progestagen levels.[39–42] Maternal plasma progestagen levels are also reported to be lower than expected in acute conditions not directly involving the fetus, such as uterine torsion and colic.[59] Somewhat surprisingly, circulating progestagen levels in the mare are actually increased rather than decreased in cases of placentitis and other situations in which fetal well-being is compromised.[57,59]

Pregnant mares also have high concentrations of estrogens in their circulation. Beginning on approximately day 40 of gestation, the primary CL and possibly supplemen-

tary CLs, under the influence of eCG, produce large quantities of estrogens.[1,4] Fetal-placental estrogen secretion begins to increase as luteal estrogen production declines. Estrogens of fetal-placental origin increase gradually until day 200 of pregnancy and then decrease very gradually until foaling.[1,4,57,59] The high levels of estrogens in pregnant mares may result in estrous behavior, and mares previously diagnosed pregnant that are exhibiting signs of estrus should be reexamined for pregnancy prior to rebreeding or performing diagnostic techniques such as uterine culture and biopsy.[1,4]

Measurements of estrogens in mares (urine or blood) beginning between days 60 and 90 of pregnancy (timing depends on assay and laboratory) can be used to detect pregnancy and fetal viability in the mare; however, pathologic conditions appear to vary in their effect on maternal estrogen levels.[1,4,57,59] After day 150 of gestation, maternal plasma concentrations of immunoassayable estrogens less than 1000 pg/ml have been reported to be associated with placentitis, and parenteral administration of estradiol derivatives has been reported to increase fetal survival rates in these instances.[57] Maternal estrogen concentrations are frequently higher than normal in mares carrying twins but are often within the normal range in pregnancies compromised by equine herpesvirus 1 infection or severe colic.[59] The effects, if any, of ergot alkaloid exposure on immunoassayable estrogen concentrations in pregnant mares are apparently inconsistent, with both increases and no discernible effects being reported in the literature.[39,42]

Relaxin is produced in large part by the placenta during equine gestation. Circulating relaxin levels increase from approximately day 75 of pregnancy until reaching a peak level at approximately gestational day 175.[1,4,59,60] These blood levels of relaxin are essentially maintained until parturition, and there is a subsequent surge in relaxin during labor.[59] Relaxin has been used as an indicator of placental health and function, and conditions suggesting placental insufficiency such as placentitis, premature placental separation, and the placental thickening seen in "fescue toxicosis" result in decreased systemic levels of relaxin in pregnant mares.[59,60]

Parturition

Parturition constitutes transport of the fetus and its associated membranes from the maternal to the external environment. The fetal hypothalamic-pituitary-adrenal axis initiates the process of fetal maturation and the cascade of endocrine and neural events that lead to parturition in the mare; however, the specific physiologic processes are somewhat different in the mare than other animal species.[1,59] CRF stimulates the release of ACTH from the fetal pituitary, and ACTH, in turn, stimulates secretion of cortisol by the adrenal glands. Elevations in fetal cortisol (fetal LH may be involved as well) appear to modulate uterofetoplacental steroid metabolism (Fig. 7-10).[1,4] Progestagens decrease precipitously approximately 24 hours prior to parturition, and, although total estrogen concentrations decrease in the maternal circulation during late gestation, specific estrogens are thought to be locally active in the uteroplacental tissues during the initiation of equine parturition.[59] Following this change in the relative influences of progestagenic and estrogenic hormones on uteroplacental tissues, several events occur that prepare the uterus for parturition (Fig. 7-10). The cervix softens, most likely facilitated by relaxin and cervical production of PGE_2. Oxytocin receptors in the myometrium increase in number, and PGF is synthesized within the uterus. Blood flow to the gravid uterus and placenta increases, allowing for adequate fetal oxygenation and nourishment, as well as a means of supplying hormones, sources of energy, and oxygenated blood to the uterus. Neural signals caused by fetal movements and myometrial contractions are involved in a complex interaction with slightly elevated basal levels of oxytocin and increased secretion of PGF to bring about the first stage of labor.[1,4,59] There is a short period of uterine quiescence during the first stage of labor, most likely due to a surge in relaxin, which immediately precedes a rapid increase

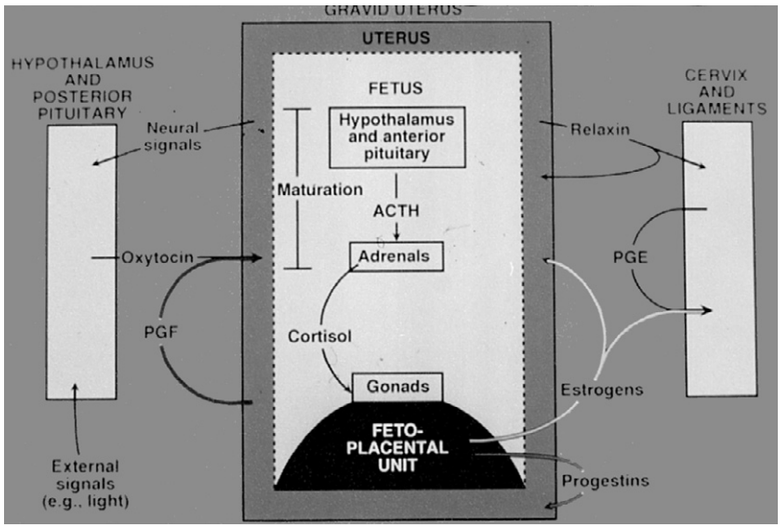

Fig. 7-10 Control of parturition. (From Ginther OJ: *Reproductive biology of the mare: Basic and applied aspects,* 2nd ed. Cross Plains, WI: Equiservices, 1992. Reprinted with permission of O.J. Ginther. Photograph courtesy of Mr. Don Connor.)

in oxytocin and PGF secretion, which leads to rupture of the allantochorionic membrane and commencement of the second stage of labor. Strong myometrial contractions result and the foal is delivered in a very short time (5–45 minutes). This myometrial activity declines after foaling but is maintained at a level sufficient to ensure expulsion of the fetal membranes (Fig. 7-10).[1,4,59,61]

The importance of the fetal hypothalamic-pituitary-adrenal axis, as well as other endocrine and neurologic pathways, in equine parturition is evident from the hormone profiles of foals, as well as mares, in a number of disease syndromes. Blood cortisol levels are decreased in premature, dysmature, and overmature (seen in "fescue toxicosis") individuals as compared to normal foals.[1,4,40,59] Decreased triiodothyronine levels in premature, dysmature, and overmature equine neonates suggest that fetal thyroid hormones may also play a role in fetal maturation and the initiation of the foaling process.[40,42] Maternal levels of prolactin, relaxin, and progestagens are all decreased in cases of "fescue toxicosis" or ergot-associated prolonged gestation, and treatment with D_2-dopamine receptor antagonists, unlike administration of rauwolfian alkaloids (e.g., reserpine), results in reversal of these endocrine trends, along with the onset of apparently normal parturitions.[41,42,62] The mare has more control over the first stage of labor than do other species, and external stimuli (such as lights and noise) can suppress oxytocin secretion and delay foaling.[1,4]

Parturition can be induced in the mare using oxytocin or a combination of prostaglandin and oxytocin. Certain criteria (duration of gestation, quality of mammary secretion, and cervical relaxation) must be met to ensure that foaling induction is safe for the both the mare and foal.[4,63] Equine parturition is a very rapid and powerful event and maternal and fetal survival depend on adequate supervision and prompt and appropriate intervention when indicated.[1,4,61]

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CHAPTER 8

Clinical Aspects of Seasonality in Mares

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SEASONAL REPRODUCTIVE PATTERNS

Natural selection would seem to logically favor a species delivering its offspring in a season of the year when weather conditions are optimal and food is abundant. Therefore, horses presumably developed their annual rhythm of reproductive competence and incompetence to ensure foals were born in spring and summer. This seasonal mechanism can thus be viewed as a timer of foaling rather than breeding. Breeding season appears timed to occur one gestation length prior to ideal foaling season.

Cueing to an unreliable environmental predictor of season could jeopardize species survival. It seems, then, that regulation of reproductive function in mares was selectively aligned to the most reliable, naturally occurring environmental cue available: photoperiod. It is important to appreciate reasons for seasonality and driving forces behind its development in order to realize that attempts to change the pattern of events quickly or acutely may not yield useful results. As a timer of foaling, this mechanism prepares 11 months or more in advance. Therefore, it is highly probable that a few days of some treatment or inappropriate weather is unlikely to change a course of events set in motion well before the breeding season. With these thoughts in mind, veterinary clinicians should consider the following phases of the annual reproductive cycle in mares.

Breeding (Ovulatory) Season

Equine breeding (or ovulatory) season is a time of reproductive competence occurring from mid spring through summer (Fig. 8-1). Nonpregnant mares experience numerous, repeated estrous cycles, ostensibly for the purpose of providing multiple successive opportunities to become pregnant. In other words, mares are seasonally polyestrous. Ovarian steroids (i.e., follicular estradiol and luteal progesterone) alternate dominance over the reproductive tract and sexual behavior in mares, setting suitable conditions for service, conception, and pregnancy.

Autumnal Transition

Transition from reproductive competence to incompetence occurs during autumn and is poorly understood compared with other annual reproductive phases. Its relative lack of economic importance compared to other phases undoubtedly accounts for some lack of interest in studying this phase. Cultural emphasis on breeding mares during late winter and early spring has made autumn-born foals less attractive in all but a few breeds. Consequently, mechanisms by which mares lose reproductive competence are not well understood, although inferences from what is known about other phases of the year allow some speculation. As mares transition to reproductive incompetence, exemplified by a relative lack of gonadotropin-releasing hormone (GnRH) and consequently reduced secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), decreasing hormones likely reflect a response to diminishing day length or some other environmental cue(s) experienced earlier in the year. As a result, a repeatable pattern of ovarian steroid production, characteristic of the ovulatory season, is interrupted, setting unfavorable conditions for sexual activity and conception. Timing of autumnal transition is highly variable among mares, occurring from September to as late as November or December. Such variability may reflect a relative lack of importance of the end of the breeding season as a selection criterion for species survival. Regardless of the reason, variability makes autumnal transition difficult to study and therefore contributes to our poor understanding of its mechanisms.

Anestrus

Anestrus is a time of complete reproductive incompetence, marked by reduction in hypothalamic GnRH content[1,2] and secretion[3] with a consequent sharp reduction in LH and FSH secretion from the pituitary.[2,4] Pituitary LH content is also dramatically reduced during anestrus.[2,4] As a result of this hypothalamic-pituitary slowdown, ovaries undergo functional, if not morphologic, atrophy with only a few follicles over 5 to 10 mm observed. Lack of follicular development is echoed by virtual loss of ovarian steroids in peripheral circulation, and progesterone and estradiol become essentially undetectable.[6–8] During anestrus, mares' sexual behavior toward stallions becomes more passive. They may not object to a stallion's presence and may even permit mounting, but intromission is often prevented by holding their tail down.[9]

Vernal Transition

Springtime (or vernal) transition from anestrus into the ovulatory season is economically and scientifically important. Desire, throughout much of the equine industry, for early-born foals conflicts with innate physiologic regulation of reproductive competence. Furthermore, vernal transition is filled with ambiguous signs that disguise the underlying process, confuse breeders, and lead to low reproductive efficiency. Owing to a lack of understanding, breeders often cover mares based solely on behavioral signs of estrus and without regard for their state of reproductive competence. On the other hand, vernal transition has been shown to follow a well-regimented series of steps leading to resumption of reproductive activity (Fig. 8–2). Vernal transition is the most precisely regulated of the annual reproductive rhythms, with relatively little variation among mares in timing of their first ovulation of the year. One of the first events in vernal transition is resumption, albeit at a reduced level compared with the ovulatory season, of GnRH secretion by the hypothalamus. Examination of hypothalamic GnRH content[1,2,10] and secretion[3] indicates the GnRH neural network increases its activity shortly after winter solstice. This increased GnRH secretion appears to be maintained throughout much of vernal transition. In response to increased GnRH, FSH secretion resumes. Though speculative, apparently the gene-regulating FSH subunit synthesis remains functional during anestrus and the lack of FSH secretion reflects a lack of GnRH secretion rather than pituitary FSH content. Nevertheless, an increase in FSH secretion is temporally associated with increased GnRH.

Ovaries, quiescent until this point, respond by developing new follicles. Follicular growth pattern during vernal transition is relatively predictable, with increased size and numbers of follicles. Of major importance, the first several follicles that develop during vernal transition generally do not ovulate, although they may reach normal preovulatory size (>30 mm). This is one of the major events contributing to reproductive inefficiency during vernal transition, as it is difficult to know whether a given follicle is competent and will ovulate. After several (3.7 ± 0.9) of these transition follicles develop and regress without ovulating, a competent follicle develops.[8] Although it is not yet known what factors contribute to eventual development of the first competent follicle, it is clear that this follicle, destined to be the first ovulatory follicle of the year, is reasonably steroidogenically competent. Development of the first ovulatory follicle is accompanied by a surge of estradiol in peripheral plasma followed a few days later by a surge in LH. The first ovulation of the year follows, ending vernal transition.

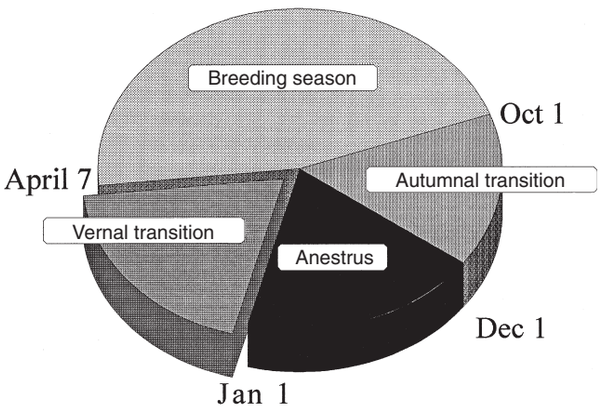

Fig. 8-1 Phases of the annual reproductive rhythm in mares. Vernal transition is emphasized because of its scientific and economic importance.

Events of Vernal Transition

1. Photoperiod response
2. Increased GnRH secretion
3. Increased FSH secretion
4. Initiation of follicular development
5. Little LH secretion = High FSH:LH ratio
6. Abnormal follicles, do not ovulate
7. Follicles do not secrete estrogen
8. Normal follicle develops
9. LH gene activated; LH secreted
10. Ovulation occurs

Fig. 8-2 Events characterizing vernal transition in mares. These events appear to be serially linked and are likely causal.

CLINICAL ASPECTS OF SEASONALITY

Challenges Seasonality Presents to Breeders and Veterinarians

A circannual rhythm in mares entrained by environmental cues ensures foaling during spring and summer. However, many breed associations observe January 1 as the official birth date of foals (in order to be eligible for registration in their respective association) born in the same calendar year. Therefore, seasonality in mares presents breeders and veterinarians with a major challenge to produce foals early. Though other environmental factors may also exert some influence on the annual reproductive rhythm in mares,[11–20] photoperiod is widely accepted as the driving force.[21] Increased photoperiod has long been recognized as a means of returning seasonally anestrous mares to reproductive competence sooner than would occur with natural day length.[22] However, increasing day length with artificial light does not shorten transition but merely causes it to begin earlier in the year.[23] Whether transition is brought about by natural or artificial light, it is a lengthy process lasting 6 to 12 weeks or more and there is considerable variation in interval from start of treatment to first ovulation among mares.[24,25] Horse breeders would benefit from a treatment or management protocol that would not only shift the beginning of the ovulatory season to a point earlier in the year but would also shorten the length of vernal transition.

Though mares in vernal transition proceed through a regimented series of steps leading to resumption of reproductive activity (Fig. 8-2), ambiguous signs characteristic of the phase are difficult to interpret and present considerable challenges for breeders and veterinarians managing transitional mares. Unknowledgeable breeders often equate behavioral signs of estrus with the ovulatory season and cover mares repeatedly during irregular and often lengthy periods of sexual receptivity. Even when follicular growth and development are monitored with behavior, difficulty in predicting the first ovulation of the year can further contribute to low reproductive efficiency.

Photostimulation of Reproductive Resurgence

In 1947, Burkhardt was the first to report early breeding of mares after doubling their daylength by adding artificial light.[22] Since then, a variety of photoexposure regimens have been used in mares to stimulate an early return to reproductive competence.[26–28] Traditionally, horse breeders exposed mares to a 16-hour fixed-length photoperiod throughout transition using a combination of natural and artificial light. Although very effective, such a long photoperiod can involve considerable expense and inconvenience. Other options available today may help breeders reduce overall expense and inconvenience of their photostimulation program. Extending natural day length with as little as 2 to 3 hours of additional artificial light was sufficient to accelerate onset of the ovulatory season.[21,24] Of interest, adding light prior to sunrise was completely ineffective, whereas adding light at sunset, thus extending the day, was very effective.[21,24] Guidelines for providing artificial light in a box stall or paddock setting have been reported.[21,29] Another effective method of providing photostimulation is the so-called "night-interruption" or "pulse lighting" approach.[30] Artificial light is applied for 1 to 2 hours during a photosensitive phase 9.5 hours after the beginning of darkness. The disadvantage of this approach is that "pulse" timing must be adjusted to changing onset of darkness or onset of darkness must be controlled artificially.[25]

Timing the start of a photostimulation program is important to effectively shift transition. Ideally mares enter the ovulatory season 1 or more cycles before intended breeding, thus eliminating the need to predict the first of ovulation and allowing ample opportunity to evaluate and prepare, if necessary, their uteri for conception and pregnancy. To effectively gain an advantage over natural photoperiod, artificial light should begin no later than winter solstice.[30] However, an even greater advantage may be possible when artificial light is applied as soon as 5 to 7 weeks before winter solstice.[24,29] One author's (GJN) clinical experience supports that assertion. Breeders waiting until January to expose their mares to increased day length will not realize any advantage over exposing them to natural photoperiod.[24,31] Traditionally it was recommended that artificial lights be continued until late spring,[28] presumably until natural day length was sufficiently long to prevent an abrupt differential in photostimulation that potentially could interfere with cyclic activity. However, it was recently reported that 14.5 hours of light applied for only 35 days beginning at winter solstice was sufficient to advance the ovulatory season.[28] The mares reached their first ovulation in a similar time as other mares exposed to an extended daily photoperiod throughout the winter and spring. Further, they remained reproductively active after artificial light was discontinued even though natural day length at the time was considerably shorter than the artificial photoperiod.

Dating back to the first report of photostimulation in mares, traditionally a 100 W incandescent light bulb, providing luminal intensity of 10 to 12 foot-candles (approximately 100 lux), has been recommended for use in a 12 × 12 box stall to stimulate onset of reproductive activity.[22,29] However, studies have investigated light intensities down to 10 lux (approximately 1 foot-candle).[21] One report indicated that light intensity of only 10 lux, when applied daily, for 14.5 hours, throughout the winter and spring, was sufficient to advance the onset of reproductive activity.[28] Little work has been done to determine the optimal spectral wavelength of light used for photostimulation in mares. Nevertheless, recognizing the eye as the primary photo receptor and maximal photochemical sensitivity being in the midrange (approximately 550 nm), it was suggested that light sources with emission ranges that include the midrange will work.[21]

Equine FSH and Pituitary Extracts

Crude equine pituitary extracts (EPE) containing eFSH and eLH have been used to stimulate follicular development and ovulation in seasonally anestrous and transitional mares.[32–34] However, EPE is not available commercially. Recently, though, a purified eFSH was used to stimulate follicular development and ovulation in transitional mares.[35] Ovulations occurred in eFSH-treated mares nearly a month earlier than control mares.

Gonadotropin-Releasing Hormone

As GnRH deficiency in mares appears to be a primary cause of reduced reproductive function during anestrus, it seems logical to administer the releasing hormone in an effort to reverse reproductive collapse. Although several studies have reported inducing ovulation in seasonally anestrous mares treated with GnRH or its analogues,[36] a number of factors limit its clinical use including cost of treatment and questions that remain regarding its preferred mode of administration (i.e., dose, frequency, route, and duration). Protocols have typically required 2 to 3 weeks of GnRH administration to induce ovulation, although a recent study induced ovulation in transitional mares with a shorter duration of treatment by repeatedly administering a slow-release subcutaneous implant containing a GnRH analogue, deslorelin.[37]

Estrogen

The positive effect of estradiol administration on LH concentrations has raised some interest and may prove to be a useful tool. However, there is an important caveat

to the use of estrogen. Although estrogen has been shown to stimulate LH secretion[4] during early vernal transition, it was demonstrated that exposure to a stimulatory photoperiod is essential for this effect.[38] Therefore, breeders who administer estrogen during anestrus or very early vernal transition might not realize any benefit from the treatment. Furthermore, estrogen treatment inhibits FSH secretion, resulting in lack of follicular development, a result contrary to resumption of the ovulatory season.

Progestins

Conflicting reports exist about the usefulness of progestins in anestrous mares. Some authors report hastening the onset of ovulation in noncyclic mares,[39,40] but others were not able to demonstrate this effect.[41–44] It appears a satisfactory response may require several conditions be met in mares selected for treatment, such as being in mid to late transition (which suggests prior photostimulation either with natural or artificial light), having considerable follicular development (i.e., multiple follicles >20 mm in diameter), and displaying estrus for 10 or more consecutive days.[39] Progestins have also been combined with estradiol 17-β; however, the suppressive effects of estradiol on follicular development delayed onset of ovulation following treatment.[45] Therefore, the combined steroid treatment did not offer an advantage over progestins alone. The results of one study suggested progestins may best be used to synchronize the first ovulation rather than hasten its onset.[41] In that case, if waiting until a mare ovulates for the first time to begin breeding management is not acceptable, breeders and veterinarians might best utilize progestins to synchronize the first ovulation and reduce the error in predicting its onset. Protocols typically call for administration of progestins for 12 to 15 days with ovulation coming within 12 to 15 days following treatment.

Dopamine Antagonists

Considerable work has recently been reported on the use of dopamine D_2-antagonists in anestrous and transitional mares. The role dopamine plays in seasonal reproductive activity and the level at which it exerts its influence are still unclear.[25] Nevertheless, several studies have demonstrated a shortened time to onset of reproductive activity in mares treated with dopamine antagonists.

In one study, seasonally anovulatory mares exposed only to natural photoperiod and treated with 1 mg/kg of sulpiride IM daily, beginning on January 30, experienced a shortened interval to their first ovulation than did untreated control mares.[46] In a second study the same workers reported treating seasonally anovulatory mares with 200 mg of sulpiride IM daily, beginning on February 5. While being exposed to a natural photoperiod, sulpiride-treated mares again experienced their first ovulation of the year earlier than control mares.[47] When domperidone was administered orally to seasonally anovulatory mares maintained under natural photoperiod at 1.1 mg/kg, they too experienced a shortened interval to their first ovulation of the year than did control mares.[48] It appears from these reports that dopamine antagonists can shorten the interval to the onset of the ovulatory season even when mares are maintained only under natural photoperiod. However, the average interval from the start of treatment until ovulation ranged from 51 to 85 days among the studies.[46–48] Additional findings indicate that prior photostimulation may improve results seen with sulpiride treatment. When mares were exposed to extended day length (14.5 hours of light and 9.5 hours of darkness) beginning on January 10 and were treated with 1 mg/kg of sulpiride IM, twice a day beginning on January 23, the mean interval to their first ovulation was 39.5 days from the start of photostimulation.[49] The interval to ovulation was shorter for sulpiride-treated mares than for their matched control mares. It is also noteworthy that the overall treatment interval required to achieve ovulation in sulpiride-treated mares exposed to an extended photoperiod was considerably shorter than had been reported in the previously mentioned studies in which mares were maintained under natural photoperiod.[46–49] In addition to extended photostimulation it also appears temperature and other environmental factors may affect the results of sulpiride treatment. Mares housed indoors and exposed to extended day length (16 hours of light and 8 hours of darkness) were treated with 0.5 mg/kg of sulpiride IM, twice a day beginning on January 18. Average interval to the first ovulation in sulpiride-treated mares was 14.8 days. The interval was shorter than that for untreated mares maintained indoors under the same photoperiod as well as that for untreated mares maintained outdoors under natural photoperiod.[50] It appears that dopamine antagonists may be most effectively employed to shorten the interval to the first ovulation of the year, when used in combination with photostimulation and moderate temperatures. It seems possible, then, to stimulate the onset of the ovulatory season in the majority of mares by mid-February even when artificial lights are not started until January.[49]

Although the body of work suggests dopamine antagonists may hasten the onset of cyclic ovarian activity in transitional mares, the mechanism is still unclear. Dopamine antagonists have been shown to cause increased endogenous prolactin concentrations in treated mares;[47,51–53] however, their reported effect on gonadotropins in horses is inconclusive.[46–48,50,53] Therefore, it is still unknown whether dopamine influences seasonal reproductive activity in mares through an inhibitory effect at the hypothalamic level or dopamine antagonists increase ovarian responsiveness to available gonadotropins through a prolactin-induced increase in FSH or LH receptors.[25]

CONCLUSIONS

Even though seasonality in mares is the result of a complex endogenous rhythm of neuroendocrine controls entrained by one or more environmental cues, we chose to limit our review to the clinical aspects of seasonality. Readers are referred to the previous edition of this text for a more in-depth review of the neuroendocrine control of seasonality in mares.[54]

The most practical tool for regulating the annual reproductive rhythm of mares remains photoperiod. It is relatively inexpensive and easy to administer. A variety of treatment regimens appear to be effective, allowing breeders choices that fit their own management schemes. Recent work suggests that combining photostimulation with dopamine antagonists may greatly reduce the interval required to stimulate the onset of the ovulatory season.

It seems clear that horses, having evolved in a temperate climate, developed a systematic, predictable schedule for mating to ensure that foaling would occur when survival of the foal was most likely. It is important to remember that the mechanisms regulating the annual reproductive rhythm had to be predictive of events one gestation length in the future. There would have been little selection advantage for regulation of the time of mating, except for its consequence. Therefore, it must be appreciated that the annual reproductive rhythm in mares is a long-term process, guided by reliable environmental conditions in a manner not yet fully understood. Attempts to alter the course of this annual rhythm must be based on a sound understanding of the underlying principles. Although several treatment regimens have shown promise in this regard, expectations of immediate response to treatments must be tempered by knowledge that the system is designed to move slowly in response to events past and in anticipation of events in the future.

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CHAPTER 9

Clinical Examination of the Nonpregnant Equine Female Reproductive Tract

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COMPONENTS OF THE EXAMINATION

History

It is essential that a thorough reproductive history be taken before a physical examination of a mare's genital tract is performed. Infertility may be the result of a previous reproductive event, such as uterine infection, dystocia, or perineal trauma, or an anatomic deficiency such as poor vulvar conformation. The history should include the number of foals the mare has produced, the years in which she has been bred, and abnormalities at parturition such as dystocia, stillbirth, or retained fetal membranes. If there is a history of retained fetal membranes, how was it resolved (oxytocin or traction, intrauterine therapy)? Is there evidence of early embryonic death, twinning or reduction of twins, or abortion? If the mare has aborted, was the cause determined (twinning, infectious agents, age, etc.)? Could abortion be related to endometrial fibrosis? It is also helpful to collect information regarding the mare's reproductive behavior and the incidence, frequency, and duration of behavioral estrus throughout the physiologic breeding season.

External Evaluation

External evaluation of mares should begin with an examination of the mammae: their size, a comparison of the halves, and the gross appearance of the teats. Attempts should be made to determine whether their appearance can be explained by the age, parity, and lactational status of the mare. Abnormalities of the perineum, including integrity of the anal sphincter and vulvar conformation, can predispose mares to aspiration of air and feces into the genital tract.[1] An assessment of vulvar conformation should include the relationship of the dorsal vulvar commissure to the pubis, the adequacy of the seal formed by the vulvar labia, and the angle of the vulva (Fig. 9-1). A wind-sucking test performed by gently parting the labia and listening for the sound of air rushing into the vagina can identify mares that would benefit from Caslick's vulvoplasty.[1] The examination must evaluate the ability of the three anatomic barriers (vulvar labia, vestibular sphincter, and cervix) to protect the uterus from the external environment and ascending infections. If a mare has foaled previously, an abnormality of the perineum may be evidence of trauma during parturition. Such mares are candidates for detailed vaginal examination to determine whether there is additional internal damage such as rectovaginal fistulae or lacerations of the vagina or cervix. The examiner should record the general appearance of the patient, taking note of body condition and conformation. If a mare has a very flat croup, she may have correspondingly poor vulvar conformation. The normal female phenotype may be altered by certain conditions such as X-chromosome monosomy, chronic use of anabolic steroids, and hermaphroditism.[2–6] If the mare is a maiden, it is important to conduct a vaginal exploration prior to first cover.

Internal Evaluation

The initial evaluation of a mare's genital tract requires a number of examination modalities, the most important of which is a skilled transrectal palpation (TRP) of the ovaries, uterine horns and body, and cervix. Ultrasonography cannot replace competent TRP, but can add information and clarify palpation findings. Manual evaluation of the cervix is an important component of a thorough vaginal examination. Digital palpation of the vagina and cervix provides detailed information on subtle changes within the vaginal vault and permits the most complete assessment of abnormalities of the external cervical os and the cervical lumen. If reproductive failure due to a cervical laceration is suspected, the examination is best conducted during diestrus when the cervix is normally closed owing to the influence of progesterone. A digital examination allows assessment of the ability of the cervix to close and protect the uterus from the external environment. An examination using a vaginal speculum may help identify tears, hematomas, abscesses, and urine pooling.

Invasive examinations of the uterus should never be undertaken before TRP, and in some cases ultrasonography of the tract has been conducted to ensure that the mare is not pregnant. Once it has been established that a mare is not pregnant, vaginal procedures can be accomplished without regard to the stage of the estrous cycle. Care should always be given to placement of a tail wrap and tail tie and thorough cleansing of the mare's anal sphincter, vulva, and perineum to minimize the potential risk of introducing contaminants.

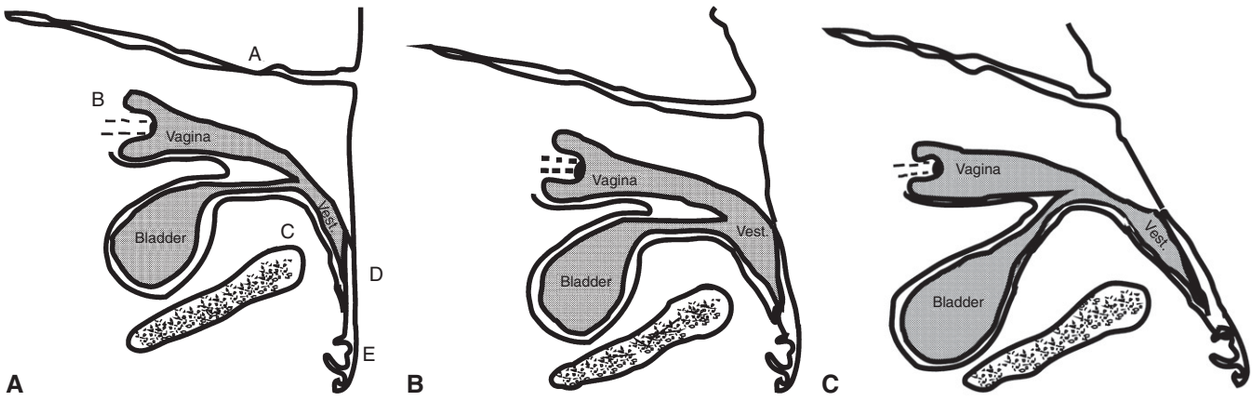


Fig. 9-1 Evaluation of vulvar conformation. Assessment includes the adequacy of the seal of the vulvae, the relation of the dorsal vulvar commissure to the pelvic floor (above, even with, or below), and the degree of forward tipping of the vulvae. The degree of tipping ranges from vertical *(Good, far left)* to an increasingly cranial slant *(Fair, middle; Poor, far right)*. Conformation changes are attributable to increasing parity, loss of vaginal fat, age-related or injury-related changes, and genetics (mother–daughter). A, rectum; B, external cervical os, C, pubis; D, vulvae; E, clitoris.

Additional means of identifying abnormalities in infertile mares are uterine cultures to determine the microbiologic status of the uterine lumen, endometrial cytologic examination to establish the presence of inflammatory cells, endometrial biopsy to detect histologic changes that could inhibit the mare's ability to sustain a fetus to term, and uterine endoscopy to identify gross lesions within the uterine lumen that might prevent conception or interfere with pregnancy maintenance. The incidence of oviductal disease is very low in mares when compared with other domestic species, such as the cow.[7] Although there have been some interesting reports that attach significance to collection of material within the uterine tubes or to inflammation of the uterine tubes, lesions of the oviducts do not appear to be common causes of reproductive failure in mares.[8,9]

Effect of Season

The reproductive activity of both mares and stallions is driven primarily by day length[10–12] and to a much lesser extent by ambient temperature. In normal mares, seasonal and environmental changes are reflected in the findings during TRP. During winter anestrus the ovaries become inactive. The weight of ovarian stroma decreases and ovarian dimensions diminish.[13] This normal change should not be mistaken for a congenital abnormality, but viewed within the context of the season. Ovarian development is a reflection of the activity, or lack thereof, of the hypothalamus and the pituitary gland. Ovarian activity (contrast inactive with the presence of follicles and corpora lutea) affects the balance of the genital tract, and in the absence of progesterone, uterine tone is poor (flaccid) and the cervix relaxed. With the coming of spring, day length increases, pineal gland suppression wanes, and activity of the hypothalamus and the anterior pituitary gland increases.[10–12,14] With consistent photostimulation (increasing day length), the size and activity of the ovaries increase dramatically.[13]

During spring (vernal) transition, there is significant follicular growth, both in size and number.[15] Transition occurs between winter anestrus and the ovulatory season (vernal transition) and again between the end of the ovulatory season and anestrus (autumnal transition).[16]

TRANSRECTAL PALPATION AND THE INTERRELATIONSHIPS OF SEASON, OVARIAN ACTIVITY, UTERINE TONE, AND CERVICAL RELAXATION

Transrectal palpation of the ovaries, uterine horns and body, and cervix is the most important skill needed by practitioners striving for success in equine reproduction. Initial evaluation of every mare must include a detailed evaluation of all components of the genital tract. Absence of a structure may suggest a congenital anomaly or may be the result of a surgical procedure (e.g., ovariectomy). After all structures of the genital tract have been identified, an understanding of their relationship to each other is essential. The examiner should establish whether ovarian activity coincides with the mare's behavior, that is, the relationship between follicular or luteal phase structures and teasing response. The correlation between uterine tone and the degree of relaxation of the cervix is also key in establishing whether a mare is reproductively normal. No portion of the genital tract can be evaluated without regard to its relationship to the whole. All recorded findings should be relayed to the client to help her or him decide on further management of the mare.

The nonpregnant uterus is T- or Y-shaped. The uterine horns approximate the cross-bar or the V of the letters. The uterine horns and ovaries are suspended by the broad ligaments (mesometrium and mesovarium) between the tuber coxae. The ovaries usually lie lateral and slightly ventral to the tips of the uterine horns. The ovaries are moveable within a range of 2 to 5 cm from the cranial border of the broad ligament and, by virtue of the mare's

motions or during palpation, may be brought to lie dorsal and medial to the broad ligament.

After fecal material has been removed and adequate rectal relaxation has been achieved, the examiner can identify the uterine bifurcation by inserting the arm approximately 30 to 40 cm beyond the cranial brim of the pelvis (generally at or slightly beyond elbow depth). With the fingers close together and slightly cupped, the arm is swept downward toward the prepubic tendon (belly wall). By bending the elbow and flexing the wrist, the examiner can lift up the uterine horns and cradle them with this gentle scooping motion. The horns are cradled within the arc formed by the thumb and fingers. Continuing further laterally, the ovaries can be individually isolated and fully palpated between a thumb and fingers. The risk to the examiner and the mare should always be kept in mind. A complete examination is frequently the result of incremental palpation. If the uterine horn findings have been recorded and a peristaltic wave is bearing down on the hand, it is better to retreat, as necessary, and continue from that point. The time lost by careful, but cautious, palpation is insignificant when compared with the value of the mare or the safety and liability of the veterinarian.

Ovarian Palpation

Structures that can routinely be palpated are follicles, ovulation depressions (OVD), corpora hemorrhagica (CH) for up to 5 to 6 days after ovulation (after which time they mature into functional corpora lutea and most are not palpable), and parovarian cysts.[17–20] During *winter anestrus* the ovaries are small and inactive, there is an absence of follicles, and ultrasonographically the ovarian stroma appears uniform and completely unremarkable. With the onset of *spring transition,* follicular activity begins. The size and number of follicles can be highly variable during this period.[21] Transition is associated with persistent follicles, which usually exhibit a slow rate of growth. Transitional follicles are anovulatory and their size and number can wax and wane.[15,21] Unlike cystic ovarian disease in cows, which is a degenerative condition, persistent follicles in mares are normal structures associated with season. Transition ends with the first ovulation and an increase in serum progesterone.[22,23] For the duration of the physiologic breeding season, mares experience an approximate 21-day cycle from one ovulation to the next.[24,25] During the physiologic breeding season, follicles are produced in waves approximately every 10 days. Generally behavioral estrus is exhibited when serum progesterone concentrations fall below 1 ng/ml and is accompanied by a dominant follicle that increases in size at a rate of 5 to 6 mm per day until ovulation.[14,26,27]

All ovulations in mares occur through the ovulation fossa; thus, developing follicles increase not only in width but also in depth as they extend through the ovarian stroma and encroach on the ovulation fossa (Fig. 9-2).[28] Measurements of follicles recorded during an ultrasonographic examination may be larger if depth is measured rather than width. Thus, consistency in records will be achieved if measurements of dominant follicle size represent the width of the follicles, not their depth.[27] Not all follicles soften prior to ovulation. As a follicle nears ovulation, it is possible to feel an irregular ridge around the perimeter of the ovulatory follicle where the ovarian stroma meets the edge of the follicle (Fig. 9-3, *D*).[19] The ultrasonographic appearance of follicular fluid is uniformly black (see Fig. 9-2, *G*).[29]

Immediately after ovulation, the distinct cavity of an OVD can be palpated (see Fig. 9-3, *E*). Although not apparent in all mares, even in mares palpated at 6-hour intervals, when an OVD is detected, its roughened perimeter is palpable level with the ovarian surface, and its deepest point extends through the ovary to the ovulation fossa. In some instances, an OVD may be delineated as long as 18 hours, but in other cases blood fills the space (CH) much more quickly. It is possible to distinguish many follicles from CH during TRP by the angle formed between the edge of the follicle or the CH and the ovarian stroma. Except for some follicles that develop at the extreme poles of the ovaries, the palpable angle between a follicle and the ovarian stroma is approximately 90 to 100 degrees.[19,27] In contrast, the angle between a CH and the stroma is much wider, 100 to 120 degrees (a CH is flatter than a follicle; see Fig. 9-3, *D* and *F*). CH also tend to be smaller than the follicle that preceded them.[3] A CH feels softer and more fluctuant than the surrounding ovarian stroma during the first 1 to 2 days after ovulation. Ultrasonographically, the CH contains echogenic particles that result from blood filling the former follicular space. As the blood clots and organizes, its ultrasonographic appearance becomes more hyperechoic and, over the span of 2 to 4 days, more uniform.[29] Although a mature CL cannot be palpated, it can consistently be distinguished by its uniform gray (more echogenic than the ovarian stroma) appearance during ultrasonographic examination (see Fig. 9-2, *K*).[19,29]

Parovarian cysts are embryologic remnants of the wolffian duct system.[3] Their only significance is that they may be confused with follicles by the novice examiner. A careful transrectal examination can more specifically identify the location of these fluid-filled structures to be only "in the vicinity of ovaries," that is, parovarian. The size of parovarian cysts increases either not at all or very slowly. They can range in size from a few millimeters to that of a modest-sized follicle.

Uterine Palpation

To demonstrate the relationship between ovarian activity and its effect on the uterus, it is necessary first to establish descriptive, user-friendly definitions. For this discussion, the definitions describing uterine tone reflect the tubularity of the uterine horns and the response of the uterine horns to slight digital pressure during TRP. The intent is to avoid the variety of numerical ranges used to define tone, as well as "food pathology" descriptions to describe the genital tract, for example, meaty, liver-like, spongy, and so on. This discussion will be based on the following definitions to follow uterine tone throughout the estrous cycle. The descriptive nature of the definitions should aid in their use and in the interpretation of TRP findings.

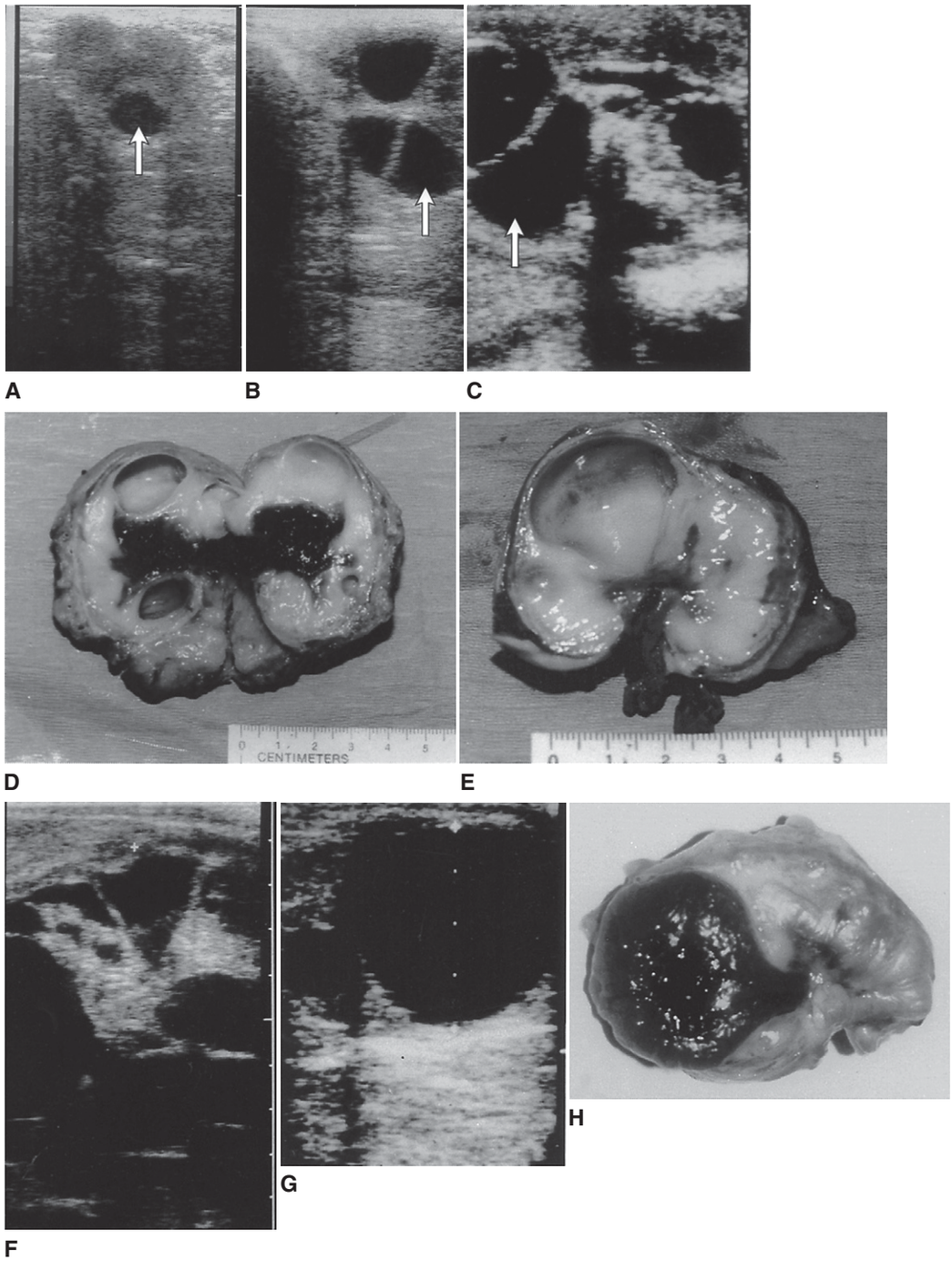
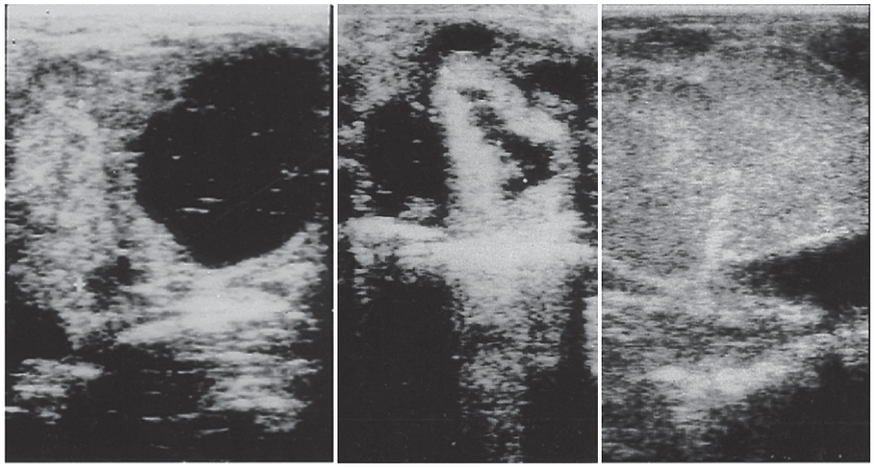


Fig. 9-2 Ultrasonographic (**A** to **C**), and cross-sectional **(D)** views of the progression of follicular development. **A** is view of a small follicle, and **D** is of a small follicle and regressing corpus luteum. The "point" of the developing follicle encroaches upon the ovulation fossa as it nears the time of ovulation. Depending on the orientation of the transducer to the ovary, this point is not always apparent during an ultrasonographic examination (**B** and **C**). **E** to **K,** Distinguishing characteristics, which can be identified in the periovulatory period, between follicles and corpora hemorrhagica: cross-sectional (**E** and **H**) and ultrasonographic (**F, G,** and **I** to **K**) views. The tonicity (firm to very soft) of ovulatory follicles and CH can be similar (**F** and **G,** large follicles; **I,** early CH). Note that **K** was a double ovulation. *Continued*.



I J K

Fig. 9-2, cont'd **I** to **K** show the progression of luteal development postovulation: **I** recent ovulation; **J,** partial luteal development; **K,** mature corpora lutea.

Excellent tone (ET): Upon palpation, the uterine horns are found to be distinctly tubular. Gentle digital indentation of the cranial ventral border of the uterine horns is met with resistance. The uterine horns feel similar to a rubber hose. ET would accurately describe the uterine tone expected in a heifer during estrus. ET in mares occurs first during early pregnancy (16–20 days of gestation) and occasionally during the first week postpartum (a reflection of optimal uterine involution). As pregnancy continues, the tone of the uterine wall immediately around the conceptus begins to decrease as the amount of fluid increases. ET may persist until 40 to 45 days from the middle to the tip of the nongravid horn.

Good tone (GT): The uterine horns are still distinctly tubular during palpation, but indentation is possible. The area of indentation readily springs back.

Fair tone (FT): The uterine horns are still tubular during palpation, but digital pressure as described above leaves an indentation that remains for variable short periods. The sensation of fair tone is similar to that of pitting edema.

Poor tone (PT): The uterine horns are not tubular, but instead flattened. On initial palpation they are found to be flaccid or atonic.

As a clinician becomes more familiar with this system, intermediate grades of uterine tone can be further defined:

Good to excellent tone (GET): Tubular and very firm, can be indented, but the indentation springs back immediately under the finger.

Fair to good tone (FGT): Tubular, springs back, but more slowly.

Fair to poor tone (FPT): The uterine horns appear initially to be tubular, but with the slightest pressure, they flatten out.

The abbreviations of tone in this sliding scale are as follows (greatest to least tone): ET-GET-GT-FGT-FT-FPT-PT.

Separate notations of tone are made for the left and right uterine horns if they differ by as much as a half grade, and separate values are recorded for diameters of the uterine horns if they differ by more than 5 mm. Individual mares may exhibit either good or fair tone during diestrus, and as they enter estrus, uterine tone begins to decrease. Typically, uterine tone decreases one grade by the time the mare ovulates. The uterus exhibiting good tone during diestrus will decrease to fair tone by late estrus. The uterus with fair tone during diestrus will decrease to poor tone near the time of ovulation. Following ovulation, uterine tone increases by one grade and returns to its former diestrual tone. In a decade of experience with 300 mares, individual mares during the physiologic breeding season tended to maintain similar "highs" and "lows" of uterine tone (GT ↔ FT, or FT ↔ PT) from one year to the next (C.L. Carleton, unpublished observations). As in any population there will be a few mares in which tone alterations between estrus and diestrus are less than one grade. Uterine horn diameter represents the average of approximately four sites between the tip (smallest diameter) and base (largest diameter) of each horn (Fig. 9-4). With practice this determination is quickly accomplished and becomes second nature for the examiner. This degree of detail can be especially useful in monitoring uterine involution in the first 8 to 30 days post partum.

Palpation of the Cervix

The dynamic capabilities of the cervix are quite extraordinary. Its proportions are influenced primarily by the presence (luteal phase) or absence (follicular phase) of progesterone. The cervix closes under the influence of progesterone.[14,17] It relaxes, to a variable degree, during the estrous cycle when serum progesterone is low, by becoming shorter and wider from the beginning of estrus up until the time of ovulation, after which time it again closes. In contrast with the cow, in which the cervix is always palpable, the palpable characteristics of the equine cervix can vary from tightly closed and easily identifiable to not palpable, if it is completely (100%) relaxed.[17,18] Consistent and accurate palpation of the cervix is the most difficult part to master of TRP of the genital tract, but persistence is worth while. To maximize accuracy of the description, cervical palpation is best left until the

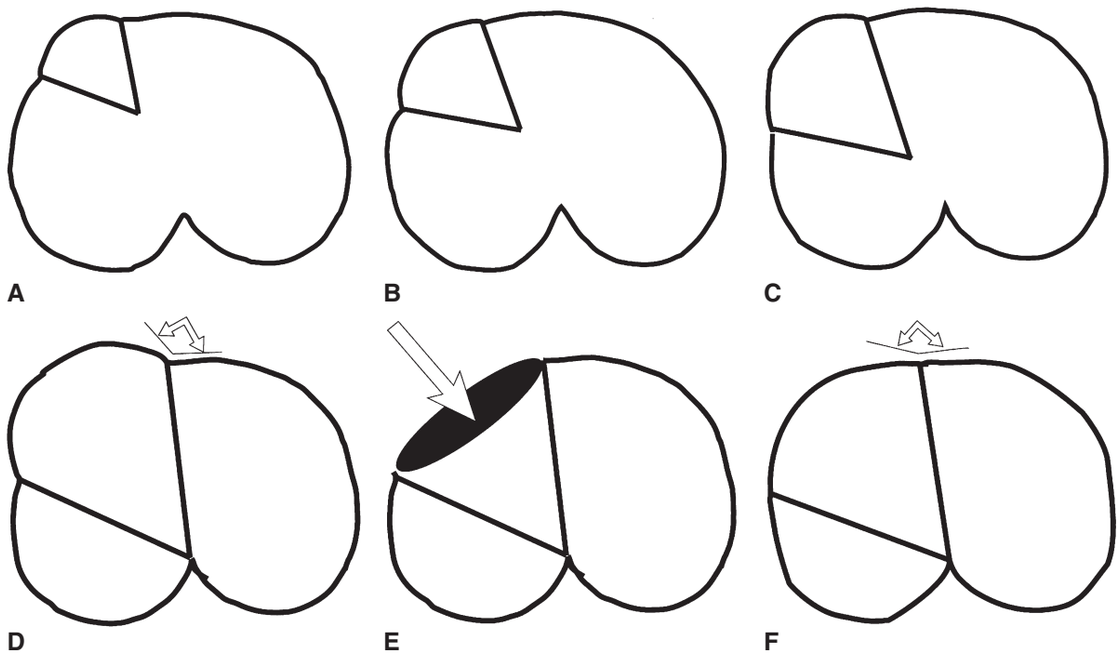


Fig. 9-3 **A** to **C,** Schematic showing the progression of follicular development. **D** to **F,** Schematic of the distinguishing characteristics, which can be identified in the periovulatory period, between follicles and corpora hemorrhagica (CH). CH are usually smaller and flatter **(F)** that the follicles that preceded them **(D)**. There is a more acute angle from the stroma onto a follicle **(D)** than onto a CH **(F)**.

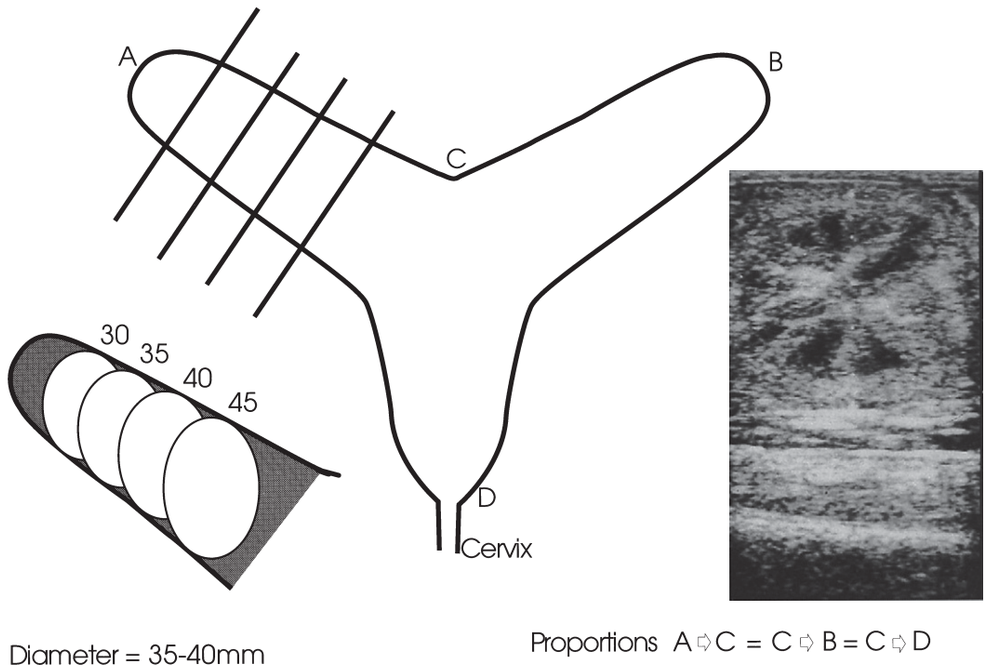


Fig. 9-4 Transrectal palpation of the uterus to establish uterine horn size (diameter). The relationship between the length of the uterine horn (A → C or C → B) and the length of the uterine body (C → D) is used as a guide to locate and facilitate palpation of the cervix. The ultrasonographic appearance of the edematous endometrial folds during estrus has been described as "orange slices" or a "wagon wheel".

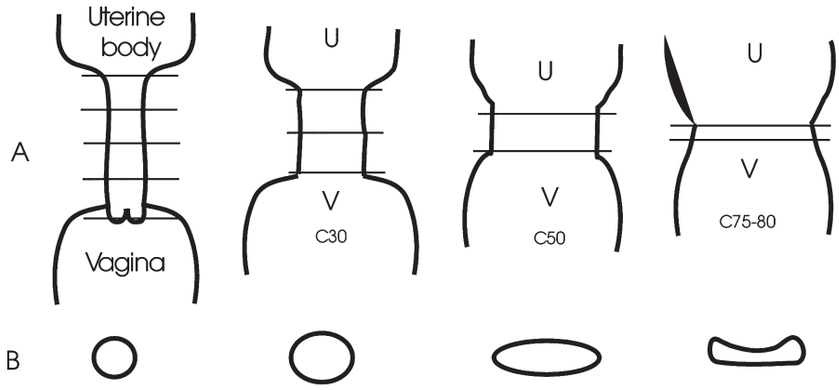


Fig. 9-5 The parameters by which assessment of cervical proportions and percent relaxation are determined (C30 = 30% relaxation). **A,** The proportion of cervical length to width; **B,** tubularity of the cervix in relation to its length:width ratio, in cross section.

Table **9-1**

Sample Transrectal Palpation Record of a Broodmare

Date	Right ovary*	Left ovary*	Uterine tone	Cervical relaxation	Teasing
April 11	F20,15	**F25**,20,15	**FT**40	C**30**	–/+
April 14†	2F20	**F35**,20,15	**FPT**40	C**40**	+
April 16†	F25,20	**F45**,25,20	**PT**40	C**50**	+
April 18†	2F25	**CH,**2F25	PT40	C**40**	+
April 20	F30,25	CH25,20	FPT40	C10	+/–

Boldface indicates *anticipated* growth and decline. See text for further explanation of abbreviations.
*Follicle (F) and corpus hemorrhagicum (CH) size given in mm.
†If records from prior seasons are available, only these palpations are essential.

end of a detailed genital examination. If the palpator has adequately described the ovarian findings and defined the diameter and approximate length of the uterine horns, then the location of the internal cervical os can readily be anticipated. The length of a uterine horn (tip to bifurcation) is approximately equal to the length of the uterine body (the distance from the bifurcation of the uterine horns to the internal cervical os) (see Fig. 9-4). As the examiner's hand is being withdrawn near the completion of a TRP, the caudal extent of the uterine body can be identified. By gently pinning of the uterine body under the outspread fingers and palm, the internal os of the cervix can be identified. There is a subtle inward coning at the transition of the uterine body to the internal os of the cervix (see Fig. 9-4). Except when the cervix is completely relaxed, the external cervical os can be identified transrectally. Using relative proportions of length to width, the closed cervix is described as having a ratio of 4:1. In the author's records, cervical relaxation is routinely recorded in increments of 10% (C.L. Carleton, unpublished observations). Full definition of the cervix requires assessment of three factors: length, width, and tubularity (Fig. 9-5). This combination provides a quick and reasonable means of assessing the degree of cervical relaxation. The percent of relaxation of the cervix is related to the structures on the ovaries. The cervix and the uterus thus are additional predictors of the mare's readiness for mating and ovulation. Most normal mares achieve at least 40% relaxation of the cervix prior to ovulation (C.L. Carleton, unpublished observations).

Prediction of the Time of Ovulation

With serial palpations, an appreciation of the relationship of a dominant follicle to decreasing uterine tone and increasing cervical relaxation can improve the breeding management of mares. Knowledge of follicular size at ovulation in individual mares from records of previous seasons and estrous periods makes it feasible to predict more closely the time of ovulation. Insemination is avoided until the follicle is within 5 to 10 mm of its anticipated ovulatory size, When coupled with evidence of decreasing uterine tone and a relaxing cervix during estrus (Table 9-1), accurate, consistent, multiple-year records are invaluable in predicting the optimal time to breed and, during subsequent ovulatory seasons, result in a decrease in the number of covers per mare. Individual mares tend to ovulate similar-sized follicles year after year. The exception occurs during foal heat, during which the dominant follicle may become significantly larger. Nevertheless, the effect of the follicle on uterine tone and on cervical relaxation is consistent near the time of ovulation. Factors that prevent the cervix from responding to ovarian activity include anabolic steroid administration, induration of the cervix subsequent to chronic pyometra, and chronic cervicitis.[4,5]

Good records permit assessment of trends and recognition of abnormalities of a mare's estrous cycle. Additional structures noted during TRP and ultrasonography may include endometrial folds (see Fig. 9-4, *inset*), uterine fluid, and uterine cysts. During estrus, the endometrial

folds are particularly prominent owing to edema.[17] Notations made of the location and size of lymphatic and glandular cysts will prevent confusion with pregnancy during an early ultrasonographic examination. If fluid is detected during TRP, its nature can be further defined by ultrasonography, microbiology, cytology, lavage, or endoscopy. An early report dealt with grading of luminal fluid and based its severity on its echogenicity.[30] The volume of uterine fluid can be estimated ultrasonographically. Few mares show evidence of gross vaginal discharge even when a large volume of fluid is in the uterus.[3] Once the presence of fluid has been confirmed, a uterine swab should be collected prior to performing other techniques because bacterial contaminants introduced during subsequent procedures could mask the original problem. Intraluminal adhesions, unless extraordinarily large or extensive, are not palpable.

Vaginal Examination

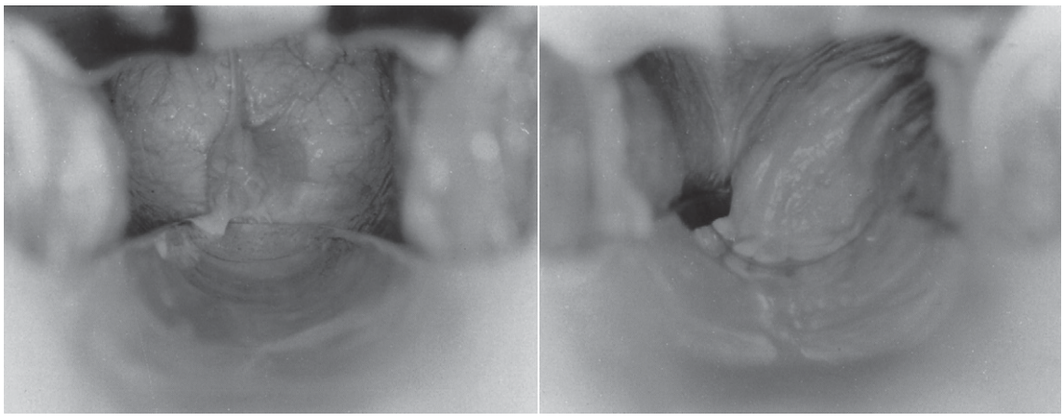
The need to perform routine vaginoscopy to assess readiness for mating should diminish as facility for cervical evaluation by TRP improves. A vaginal examination is a standard part of a breeding soundness examination and is an essential part of the evaluation of a subfertile mare or whenever discharge is noted at the vulvar lips. A *digital vaginal examination* is better suited than vaginoscopy to detect and evaluate subtle irregularities of the mucosa caused by trauma, as well as more obvious adhesions. To rule out cervical tears, it is important to assess cervical integrity during diestrus.[31] With an index finger in the cervical lumen and the thumb on the outside of the external os, it is possible to discern subtle abnormalities of the cervix. The integrity of the cervical lumen can be determined by exploring it for areas of thinning, tears, and adhesions. A digital vaginal evaluation prior to the first live cover or artificial insemination will determine if a persistent hymen is present. The hymen may be imperforate, or only remnants may remain.[4,32] In either instance, the hymen should be eliminated 10 to 14 days prior to breeding to avoid hemorrhage at the time of cover.

A speculum examination can help to further define lesions detected during a digital examination. A *vaginal speculum* requires a bright light source to fully evaluate the vaginal vault. A vaginal speculum examination may aid in determination of the origin of exudate (which may stem from the uterus, cervix, or vagina) noted at the vulvar lips.[19] If an assessment is made immediately upon placement of the speculum into the vagina, a vaginoscopy permits an accurate evaluation of vaginal color. The mucosa is pale pink to salmon in color and glistens during estrus.[33,34] During diestrus, the vaginal walls appear dry and pale. The mucosa rapidly becomes hyperemic following distention of the vagina with air. As the cervix relaxes during estrus, its position drops from the center of the cranial face of the vagina toward the cranial vaginal floor. After ovulation, the cervix closes and regains its former location (Fig. 9-6). Fecal matter may gain entrance to the vagina through a rectovaginal fistula or may be present because of poor vulvar conformation and pneumovaginitis. Dystocia may result in vaginal abscesses and tears.[35,36] A speculum is used to identify mares suspected of urovagina.[37,38] Abnormalities infrequently seen include enlargements of Gartner's ducts, vaginal fibromas, and venous varicosities.[3,39]

ADDITIONAL TECHNIQUES FOR EVALUATION OF INTERNAL UTERINE HEALTH

Culture

A *uterine culture* is an essential tool to determine the etiology of uterine infections.[40,41] A swab is the most accurate means of obtaining samples for identification of the specific bacteria that cause infection. A complete microbiologic work-up includes identification and antibiotic sensitivity of the bacteria and is essential to developing a specific treatment plan. Endometrial biopsy and cytologic



A B

Fig. 9-6 The appearance of the external cervical os during diestrus (closed) **(A)** and estrus (relaxed) **(B),** as viewed through a vaginal speculum.

examination may identify the presence of bacteria or fungal elements or inflammatory cells, but do not identify a specific etiology. A uterine culture is an essential component of a prepurchase examination of a broodmare and may be required prior to presentation of a mare at a breeding farm. Endometrial culture is a routine procedure in the evaluation of mares mated unsuccessfully at two or more estrous cycles during the physiologic breeding season. Additionally, endometrial cultures provide essential information for the evaluation of mares that suffer dystocia, give birth to a weak or dead fetus, experience retained placenta, and are barren at the end of the season.

Technique

To avoid collecting poor-quality swabs and to minimize contamination of the vulvar lips, it is essential to wrap the mare's tail and secure it to one side. The perineum is washed meticulously, including the anal sphincter, the vulvar lips, and the surrounding perineal area. Use of sterile equipment to collect the sample (lubricant, sleeves, and guarded swabs) is mandatory.

Sample quality is improved by the use of a guarded swab. Its quality is further improved by the practitioner's adequately protecting the tip of the guarded swab in the palm and holding it securely under the thumb as it is carried through the vulvar lips, vestibule, and vagina. The cervical lumen is not always straight. Insertion of the index finger through the cervical canal facilitates passage of the guarded swab and reduces the time required to traverse the cervix.

Samples obtained by introduction of a swab through a vaginal speculum frequently become contaminated. The swab cannot consistently and efficiently be directed from the cranial vagina through the cervical lumen. It must be advanced cranially to a point at which one must assume the uterine body has been reached. Resistance within the cervical canal may falsely be interpreted as the swab having come into contact with endometrial folds. Samples obtained through a speculum are of questionable quality and their interpretation is suspect.[3,43,44]

Microbial Flora in the Genital Tract

The number of bacteria in a mare's genital tract decreases cranial to the vulva, with diminishing numbers in the vestibule, vagina, and cervix.[44] There are no normal resident microbial flora of the uterus.[3,45–47] There are reports suggesting the presence of a normal uterine microbial flora, but it is more likely that mares suffering chronic infertility have persistent uterine contamination or infection or both. Bacteria are usually introduced into the uterus during breeding, during foaling, or during other invasive procedures.[3] It is essential that the sample for culture be taken from the uterus and not the cervix. Isolates recovered in pure culture (single organism) are more significant. Ideally the laboratory will determine not only the identity of the organism but the number of colony-forming units as well. Recovery of bacteria from endometrial swabs can be enhanced by gently advancing the swab cranially and allowing it to remain in contact with the endometrial folds for at least 30 seconds, which improves absorption of uterine fluid into the swab.[45] Isolation of more than two bacterial species from a uterine swab often indicates poor collection technique, but may also reflect ascending contamination of the uterus secondary to poor vulvar conformation and pneumovagina. A guarded uterine horn lavage (UHL) technique was developed that was compared with other sampling methods.[45] Seventy-five percent of all aerobic endometrial samples from normal mares collected using UHL yielded no bacterial growth, compared with 35% of those collected using a standard guarded swab. In this project, the few bacterial isolates from both UHL and endometrial swabs were all common contaminants. The numbers of colony-forming units were considered to reflect the rigor with which the mare was prepared for the procedure as well as the degree of protection from contamination provided by each technique and did not reflect a normal uterine microbial flora.[45]

The uterus of normal *resistant* mares has been shown to be able to eliminate bacteria within 96 hours after inoculation.[48] Other mares are unable to overcome bacteria introduced at the time of breeding or foaling and are described as *susceptible* to contamination and reinfection.[48,49] One manifestation of reinfection is luminal fluid identified during TRP and ultrasonographic examination of such mares. Oxytocin has been reported to be a useful treatment to reduce fluid in the uterus at the end of estrus following breeding of subfertile mares.[50–53]

With few exceptions, barren mares are presented for a breeding soundness examination without the luxury of the stage of their estrous cycles being known. There are conflicting opinions concerning the practice of obtaining samples for endometrial culture only during estrus when uterine resistance is greatest and the defense mechanism is most effective in clearing contamination.[54,55] This response is diminished during periods of progesterone dominance, and the number of bacteria, immunoglobulin A, and nonantigenic markers remain elevated for prolonged periods after inoculation.[56,57] If it has been determined that the mare is not pregnant and if preparation of the mare is meticulous and considerable care is taken to minimize introduction of contaminants, endometrial samples for bacterial culture can be taken during either estrus or diestrus. Dilation of the cervix, even when closed during diestrus, is simply accomplished and causes no harm to the mare.[58]

Septic metritis is an infrequent finding in postpartum mares. It is an acute condition and is characterized by depression, anorexia, fever, and laminitis. Predisposing factors include retained fetal membranes, dystocia, and a grossly contaminated foaling environment. Unless a mare is acutely ill, uterine cultures taken during the first week post partum are unlikely to be very useful, especially if taken prior to foal heat (10 days post partum). Isolation of multiple bacterial species and large numbers of colony-forming units reflects contamination introduced during normal parturition.[59] For breeding farms to require a clean, "no growth" culture of mares presented for foal heat breeding is generally an exercise in futility.

Significance of Bacterial Growth

The majority of pathogenic bacteria isolated from uterine swabs are β-hemolytic streptococci *(S. zooepidemicus, S. equi,* and *S. equisimilis).*[44] Other significant pathogens are *Klebsiella* species, *Pseudomonas* species, *Candida* species, and hemolytic *Escherichia coli.*[60] Some laboratories support typing of *Klebsiella* isolates to determine their pathogenicity, but this has not gained wide acceptance.[61] Nonhemolytic *E. coli* is the most common uterine contaminant and with few exceptions is a reflection of poor vulvar conformation and windsucking. Following Caslick's vulvoplasty, *resistant* mares clear bacteria within one or two estrous cycles and do not require treatment with antibiotics.[49] *Staphylococcus,* β-hemolytic streptococci, *Gardnerella,* and other enteric organisms are common contaminants.[62] Uterine infections caused by yeasts and molds commonly follow inappropriate or excessive antibiotic therapy.[63,64] Identification and antibiotic sensitivity testing of isolates are essential to select a proper treatment regimen.

Equipment

A number of guarded instruments are available for obtaining endometrial samples for bacterial culture, including Kalayjian (Kalayjian Industries, Inc., Long Beach, Calif.) and Priority Care (First Priority, Inc., Gilberts, Ill.). Combination rods (Accu-CulShure, Accu-Med Corp., Pleasantville, NY.) are available and can be used to collect a sample for microbiologic culture as well as a sample for endometrial cytologic examination. A cytologic examination can also be performed using the cells collected within the cap of the Kalayjian rod during collection of the bacterial culture.[58]

Most commercially available guarded uterine swabs do not incorporate transport medium within their design. If a sample cannot be immediately streaked onto a selective medium and blood agar, then the swab should be placed into an appropriate transfer medium and refrigerated until it can be processed.[43] One usable system in which a swab can be transported is the Culturette (Becton-Dickinson Microbiology Systems, Cockeysville, Md.). The swab packaged within the Culturette is similar in length to the detachable swab at the tip of the majority of uterine culture rods. The Culturette's swab is discarded and the swab from the guarded culture instrument takes its place. The fluid-filled ampule is crushed to prevent desiccation of the swab. The Culturette is refrigerated until the sample can be placed on artificial medium and incubated.

Therapy

If culture results indicate the need for therapy, uterine lavage can be useful to reduce fluid collection in the uterus. The efficacy of most antibiotics is diminished in the presence of debris.[65,66] Lavage, followed by instillation of an antibiotic, increases the efficacy of treatment by increasing contact of the antibiotic with the endometrial folds. Lavage also provides a clinical gauge of myometrial responsiveness.[67] The author has experienced greater success in treating and achieving pregnancies in barren mares from which at least a 75 to 80% return of the lavage solution was obtained. Serial lavage continues until the efflux is clear (Fig. 9-7). Appropriate antibiotics, selected on the basis of a sensitivity test, can then be instilled in a volume proportional to uterine size.[66]

Endometrial Cytologic Testing

The significance of cytologic examination is a matter of debate among theriogenologists.[69,70] Reports published during the early 1980s recommended taking samples for bacteriologic and cytologic testing during early estrus, 15 to 17 days after the previous ovulation.[42] The intent was to obtain a sample before the uterus came fully under the influence of estradiol and the "increasing bactericidal capacity of the uterine secretions." Circulating neutrophils are recruited to the uterine lumen in response to antigenic material. Their presence is an indication of an acute and active inflammatory process. The usefulness of cytologic testing may be greatest in the mare from which few, but pathogenic, bacteria are isolated. Small numbers of organisms not accompanied by an inflammatory response are less likely to be significant or require treatment.[68,71] Cytologic testing may be utilized late in the breeding season if there is pressure to make treatment decisions before results of uterine cultures and biopsies

Fig. 9-7 Significant amounts of luminal fluid in the uterus preclude effective treatment with antibiotics. A uterine lavage reduces fluid and debris. A successful lavage continues until the color and nature of the return fluid is like that of the instilled medium (saline): left to right.

are available. Although the presence of inflammatory cells is significant, their importance must be kept in perspective. By itself, cytologic testing is not a means to identify the etiologic agent.[31,70]

There are a number of ways in which a cytologic sample may be obtained. Following collection of the microbiologic sample, a second guarded culture swab can be introduced through the cervix and rolled against the endometrium and the swab then gently rolled along a glass slide. If a Kalayjian culture swab has been used, a cytologic sample may be collected from cellular material and fluid that become entrapped within the unseated cap of the swab.[58,60] Recovery of cells can be maximized as the instrument is withdrawn from the uterine body by rotating the swab to facilitate a pick-up of cells from the endometrium. Culture and cytologic instruments have been designed specifically for dual sample collection. Cells can be retrieved with a syringe and pipette by injecting and aspirating a small volume of saline in the uterine body.[72] Curettage was used as a method of treatment for barren mares. A similar or modified technique could serve as a method of harvesting material for a cytologic examination.[73,74]

It is an indication of inadequate sampling of the endometrium if epithelial cells are not present in the sample, and a second cytologic procedure should be done. The appearance of epithelial cells may range from columnar to cuboidal, depending on the stage of the mare's estrous cycle. In addition to epithelial cells and neutrophils, other cells present may include lymphocytes, monocytes, eosinophils, red blood cells, and, after breeding, spermatozoa.[72,75] The speed with which inflammatory cells enter the lumen following any intrauterine procedure must be kept in mind.[47] Normally there are no neutrophils in the lumen.[76] If a cytologic sample has not been collected simultaneously with the sample for bacterial culture, then it should be collected immediately thereafter. If there is a delay, then the significance of the recruited cells may be doubtful. As in endometrial biopsy specimens, lymphocytes, monocytes, and macrophages are usually a reflection of chronic endometritis.[58] Eosinophils are infrequent and are most likely associated with urine pooling and chronic windsucking. Interpretation can be complicated if neutrophils are present without accompanying bacteria. Significance is based in part on the frequency of polymorphonuclear cells (PMNs) observed, that is, when the ratio of epithelial cells to PMNs falls below 10:1.[59] As the number of PMNs increases, fertility decreases.[77] UHL was also compared with standard cytologic technique. The UHL collection technique caused the least disruption to the integrity of cells retrieved. Unlike a standard cytologic sample, UHL revealed no difference in the nucleated cell counts recovered from normal mares in estrus, diestrus, and anestrus.[45]

Interpretation of the cytologic sample is easier when the sample is examined promptly on a wet mount. Diff-Quik (American Scientific Products, McGaw Park, Ill.) stain, with a three-stain procedure, is easy to use.[58] Gram stain is an easy screening stain for the presence of bacteria.[75,78,79] New methylene blue can help identify capsular material of potential pathogens, such as *Klebsiella*.[80] If the laboratory capability allows, a cytospin can facilitate concentration and evaluation of samples that contain low numbers of cells.[45] An aerosol cytofixative (Spray-Cyte, Clay-Adams, Parsippany, NJ) can be sprayed on the sample to prevent cellular distortion that occurs with drying.

Endometrial Biopsy

Endometrial biopsy is an important key to identifying the nature of an infertility problem. When combined with the results of TRP, ultrasonography, and microbiology, it permits a more accurate assessment of a mare's reproductive prognosis. Factors have been defined that provide a prognosis for the mare's fertility.[71] These factors include inflammation, periglandular fibrosis, cystic glandular degeneration, and lymphatic stasis. Candidates and circumstances for which endometrial biopsy is warranted include barren mares bred repeatedly to a known fertile stallion; mares presented for genital surgery (rectovaginal lacerations and cervical tears) that may have already suffered permanent endometrial damage; mares with a history of early embryonic death, abortion, or retained fetal membranes; mares failing to cycle during the physiologic breeding season; or as part of a prepurchase examination of a potential broodmare.

Endometrial tissue is readily obtained with a biopsy punch (Pilling, Fort Washington, Pa.). Ideal sample size for interpretation is at least a 10 to 20 mm × 3 mm sample.[81] Both the epithelial cell layer and the glandular architecture reflect season of the year and the stage of the mare's estrous cycle.[82] The luminal epithelium peaks in height from 30 to 40 μm during early estrus, then decreases to 15 to 20 μm by late estrus or early diestrus. It increases once again slowly through diestrus into early estrus. The luminal epithelium appears atrophic during winter anestrus and is characterized by cuboidal epithelium and straight, rather than tortuous, glands.[81] For proper interpretation, the results of TRP and stage of the estrous cycle should accompany the sample at the time of submission.

Technique

A biopsy can be taken by either a rectovaginal method[71] or a vaginal approach.[83] With the rectovaginal method, once the mare has been properly prepared, the sterile biopsy punch with its jaws closed is carried in a gloved hand through the vagina, gently guided through the cervical canal, and positioned in the uterine body. The hand is then withdrawn from the genital tract and placed in the rectum. The punch is palpable within the uterine body and is advanced to the desired biopsy site. The jaws of the punch are opened and rotated until the flat edge of the biopsy basket is palpable under the fingers as an opened V shape. With slight digital pressure, the endometrium is pushed into the V and the handle of the instrument closed to excise the tissue sample. In the vaginal technique, the closed instrument is carried through the cervix and advanced 2.5 to 3 cm beyond the tip of the index finger into the uterine body. The tip of the biopsy punch is advanced with the basket opened an additional 4 to 6 cm. With the index finger in the cervix

as a fulcrum, the punch is swung laterally to move the tip into the endometrial fold. In this technique, when the veterinarian's right arm is in the mare, the biopsy is most easily obtained from the left side of the lumen; conversely, the left arm in the mare's genital tract makes right-sided sampling easiest. The jaw is closed abruptly and the instrument withdrawn. It is readily apparent if a tissue sample has been obtained as the endometrium trapped within the basket will be tugged caudally and palpated by the tip of the index finger. With a gentle tug, the instrument is withdrawn.

The specimen should be gently teased from the basket with the use of a small sterile needle and placed in either Bouin's solution or 10% buffered neutral formalin.[84] The fixed tissue is trimmed, placed in paraffin blocks, and sectioned. Sections are routinely stained with hematoxylin and eosin. Neutrophils are associated with an acute inflammatory response while lymphocytes, monocytes, and plasma cells serve as indicators of chronic inflammation. An interpretation should indicate the location of the inflammatory cell infiltrates within the lamina propria, for example, stratum compactum (immediately under the epithelial cells, nearer the lumen) or stratum spongiosum (deeper, vicinity of gland branches).[71] Antibiotics and uterine lavage, used appropriately, appear able to reverse endometrial inflammation to some degree.[54,65] Fibrosis is most commonly identified in a periglandular location, and its severity is gauged by the number of layers encircling glands, as well as by the frequency of nesting of groups of glands in a more extensive response (Fig. 9-8). Fibrosis usually occurs secondary to chronic inflammation, and glands lose their ability to function.[71,85] Significant loss of uterine glands appears to be associated with inability to sustain pregnancy beyond the first few months of gestation.[71] There is no known treatment that can reduce fibrosis. Cystic glandular degeneration is usually secondary to severe periglandular fibrosis. Dilated lymphatics can be distinguished from cystic glands by their lining of endothelial cells. Extensive lymphatic stasis can interfere with fertility, and some improvement has been reported following a series of hot saline uterine lavages.[71] Incidental histologic findings can further define ongoing problems or historical events. The presence of siderocytes suggests an episode of intrauterine hemorrhage sometime during the previous 7 months and may be evidence that either an abortion or parturition occurred.

Interpretation

Interpretation of endometrial biopsies provides an estimate of both the frequency and distribution of inflammation and fibrosis, as well as the prognosis for the mare's ability not only to become pregnant but to carry a pregnancy to term. Based on the interpretation, the endometrium is assigned to one of four categories: I, IIA, IIB, or III.[71] The higher the number, the poorer the prognosis.

Category I includes mares that have minimal or no changes in endometrial architecture (Fig. 9-9). These mares have a 70% or greater chance to conceive and to carry a fetus to term. *Category II* endometria exhibit changes that diminish the mare's ability to conceive and carry a fetus to term. The nature, frequency, and distribution of architectural changes will place the biopsy into either a IIA or IIB classification. Any of the factors previously described may be involved, singularly or in combination, such as slight to moderate inflammatory cell infiltration of the stratum compactum, scattered but frequent foci of inflammation and fibrosis throughout all areas of the sample, scattered periglandular fibrosis of gland branches, or nesting of glands up to an average of three per 5.5 mm in at least four fields, or widespread lymphatic stasis noted only by biopsy.[18] Mares with predominantly inflammatory changes (IIA), and cases for which treatment may be beneficial, fare better than those with predominantly fibrotic changes. The latter, for which treatment is either unavailable or which have moderate to severe inflammation and severe fibrosis, are classified as IIB. Correspondingly, the reproductive prognosis for carrying a fetus to term diminishes and is approximately

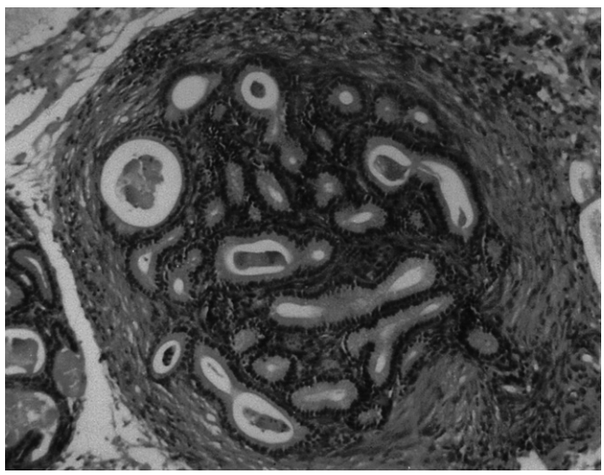


Fig. 9-8 Endometrial section with a fibrotic glandular nest and cystic degeneration subsequent to the fibrosis.

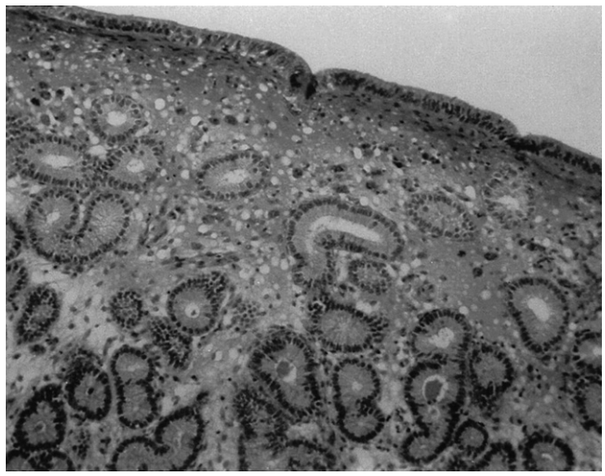


Fig. 9-9 Endometrial section with normal gland distribution and frequency.

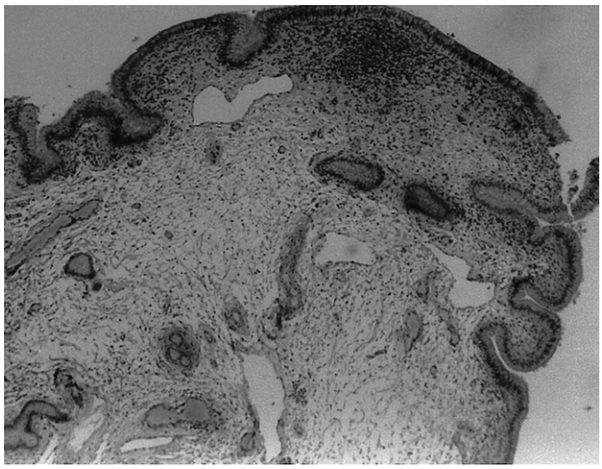


Fig. 9-10 Category III endometrial section lacking normal glands and with a locus of inflammatory cell infiltration in the stratum compactum.

50 to 70% in mares with IIA endometria and 30 to 50% in endometria classified as IIB. Category III endometria exhibit widespread or severe irreversible changes that negatively affect both conception and the mare's ability to maintain a pregnancy to term (Fig. 9-10). Examples include extensive periglandular fibrosis (five or more nests per low power field), widespread and severe inflammation, severe lymphatic stasis that results in a spongy feel to the uterine wall (grossly palpable), chronic pyometra associated with atrophy of the endometrial folds or pervasive inflammation, or endometrial atrophy that persists throughout the physiologic breeding season.[18] Such mares have a less than 10% chance of conceiving and carrying a fetus to term. The expense of attempting to get one more foal from such a mare must be weighed against the value of the mare and her potential offspring.

The cause of uterine infection may be suggested if numerous bacteria or fungal elements are evident in a biopsy sample.[71] Additional sections can be cut from the tissue block and stained to attempt identification of specific bacteria (Gram's stain) or fungal elements (methenamine silver stain).[75,78,79,86]

Endoscopy

Endoscopy is not often used during an initial breeding soundness examination, but is generally reserved as a second tier of examination of mares in which the cause of infertility remains undefined. It is also used to define more completely those abnormalities identified by TRP or ultrasonography. A flexible endoscope permits visualization of the vagina, external cervical os, and lumen of the uterine body and horns up to the uterotubal junctions.[87] During endoscopy, gross lesions can be defined, site-specific biopsies obtained from abnormal-appearing uterine mucosa, and samples of uterine fluid aspirated. Depending on the number and diameter of channels of an endoscope, specialized instruments are available that allow observation of and access to lesions. Available instruments permit site-specific biopsy (though the sample size is very small), aspiration of uterine content, curettage, and laser-guided surgery.

Dilatation of the uterine lumen with either air or fluid may cause discomfort, and problems can be averted by sedating the mare prior to performing the procedure.[87] If fluid is used to dilate the uterine horns, it can be introduced either through the channel of the endoscope or through a separate uterine infusion catheter. Infusion through a catheter is particularly helpful if it is anticipated that large volumes will be required to achieve adequate distention. Endoscopy is more easily accomplished when a mare is in diestrus. A closed, diestrual cervix slows the escape of air or fluid through the cervix and thus facilitates dilatation of the uterine lumen to permit visualization of gross lesions. During estrus or anestrus, when the cervix is relaxed, it is more difficult to distend the uterus because fluid escapes through the cervix.

Sufficient distention may be achieved solely using air in combination with the reservoir attached to the biopsy flush channel. The reservoir can be filled with sterile distilled water. Abnormalities may be difficult to identify if there is exudate or fluid in the uterus. The flush channel can be used, as needed, to remove exudate from the head of the endoscope as it is advanced into the uterus. Cloudiness caused by a mix of flush solution and uterine contents may mask subtle lesions, such as mucosal color changes or small cysts. If the amount of uterine fluid is large, it may be necessary to precede endoscopy with uterine lavage. In this instance, in exchange for better visualization of gross lesions such as transluminal adhesions and cysts, the examiner may be unable to distinguish between hyperemia or inflammation caused by lavage and that associated with infertility.

Particular attention must be paid to preparation of equipment prior to use to avoid contamination of the uterus. Cold sterilization of the endoscope, using either glutaraldehyde (Cidex, Johnson & Johnson Medical, Inc, Arlington, Tex.) or ethylene oxide (EtOH, 3M Animal Care Products, St. Paul, Minn.), is essential. The endoscope channels must also be disinfected to eliminate an additional source of uterine contamination during introduction of flush solution or instruments. This may be accomplished by immersing the flexible portion of the endoscope in a tray filled with glutaraldehyde and aspirating the solution into the channels with 60-ml syringes attached to the proximal ports of the endoscope. Although it is important to comply with the minimum contact time recommended by the manufacturer, the suggested time should not be exceeded. The endoscope and its seals can be damaged by prolonged immersion. Glutaraldehyde is very irritating to both skin and mucous membranes. Upon removal from the disinfectant solution, both the external surface and the channels of the endoscope must be thoroughly flushed with sterile water to avoid placing irritants into the genital tract. One assistant should be available to handle the flexible portion of the endoscope. Aseptic procedures should be followed from the time the endoscope is removed from the disinfectant or the sterile package (if EtOH was used) and throughout placement in the genital tract.

Gross Pathology and Endoscopy

The examination begins as soon as the tip of the endoscope is carried through the vulvar lips. A detailed examination includes observation of the gross appearance of the vestibule, vagina, external cervical os, and the uterine body and horns. The uterine mucosa should be uniform in color (a pale salmon color) and glisten (Figs. 9-11 and 9-12). To avoid confusion between the bifurcation of the uterine horns and a transluminal adhesion, attention should be paid to the length of the endoscope as it is introduced into the uterus and note made of the depth at which the bifurcation of the uterine horns is first observed (Fig. 9-13). Transluminal adhesions can range from very thin and fibrinous in nature, to thick bands, to complete occlusion of a uterine horn. Patience is essential for careful and complete examination of the uterine body and both horns. Identification of the ostium, the small papilla at the apical end of each uterine horn, provides assurance that the entire length of each uterine horn has been traversed (Fig. 9-14). Lymphatic cysts are common in older mares.[18] Their number and size tend to increase with age and they may be pedunculated or broad-based and multilocular.[71] Lymphatic cysts more frequently impinge on the uterine lumen and may be large enough to impede transuterine migration of the

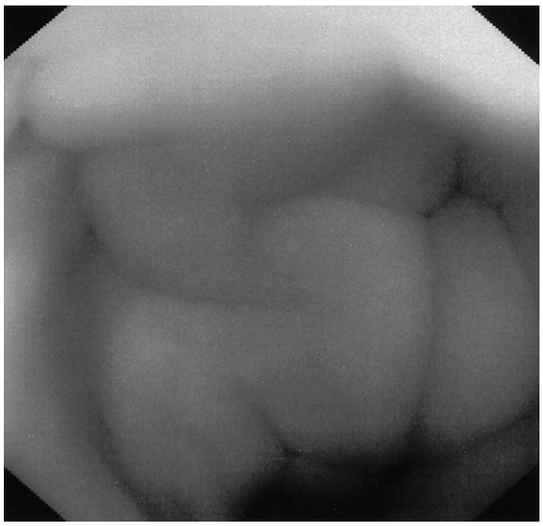


Fig. 9-11 Endoscopy of the uterus: early in the procedure, with prominent endometrial folds and minimal dilatation and expansion of the lumen.

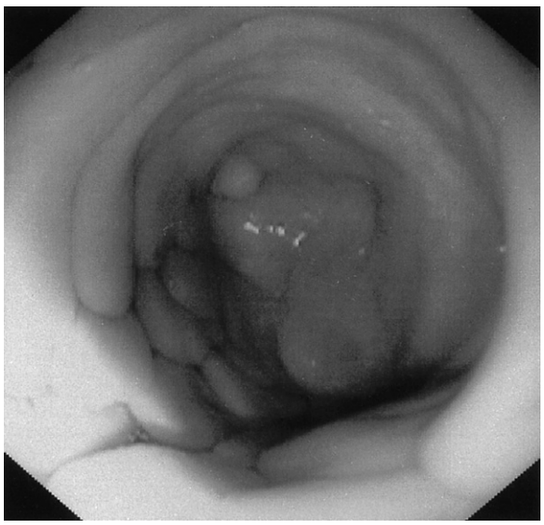


Fig. 9-12 Endoscopy of the uterus: partial dilatation.

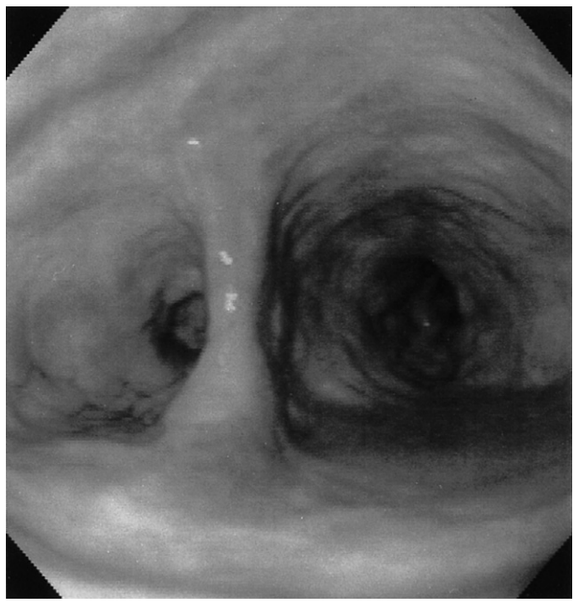


Fig. 9-13 Endoscopy of the uterus: full expansion with concomitant loss of endometrial fold architecture (which recovers upon removal of the fluid or air) and identification of the bifurcation of the uterine horns.

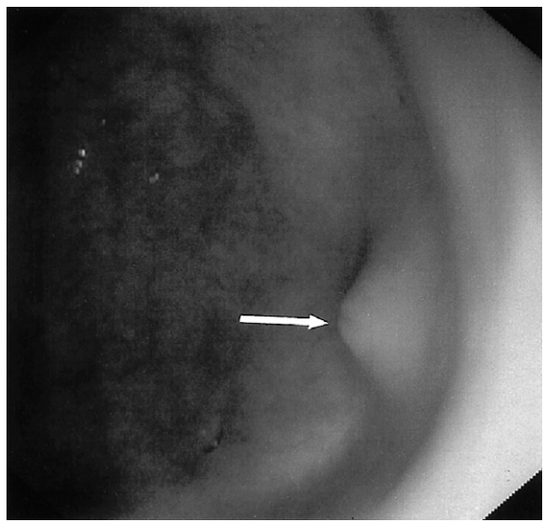


Fig. 9-14 Endoscopy of the uterus: to confirm passage throughout the full length of each uterine horn, the ostium (uterotubal junction, *arrow*), should be identified at the tip of each uterine horn.

embryo during early pregnancy. Erosions of the uterine wall, exudate, and color changes of the endometrium can be identified. The appearance of the endometrial folds may be misleading if observed during anestrus or vernal transition, as they will appear underdeveloped or atrophic.[18]

Resolution of Problems Diagnosed by Endoscopy

Lymphatic cysts can be very large and occlude the uterine lumen (Fig. 9-15). A variety of techniques have been used to remove them or reduce their size. Methods of cyst reduction include partial removal of the cyst wall with an endometrial biopsy punch, digital removal of pedunculated cysts,[54,58,88] or laser surgery to remove a large portion of the wall of a broad-based cyst (Fig. 9-16). The laser (Nd:YAG contact laser, Surgical Laser Technologies, Oaks, Penn.) is introduced through the biopsy channel of the endoscope, which permits careful and measured ablation of the cyst wall. These procedures may be followed by uterine lavage to remove the contents of the collapsed cysts. If an insufficient portion of the wall is removed, some cysts will seal and refill.

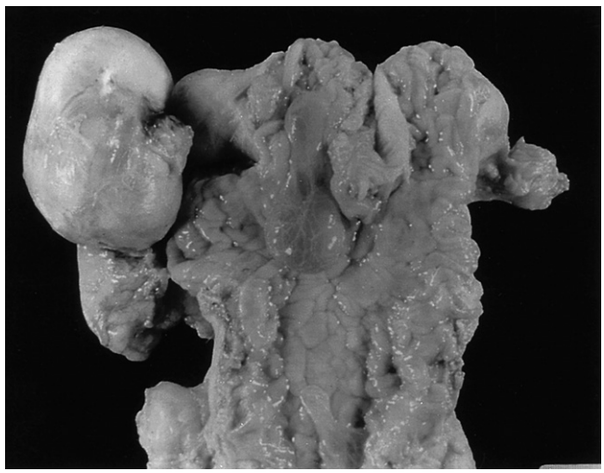


Fig. 9-15 Large lymphatic cyst occluding the lumen of a uterine horn. Note also that this mare consistently ovulated only on the side of the lymphatic cyst, which prevented transuterine migration of the embryo.

Additional gross changes observed during endoscopy may be the result of chronic infection, scars secondary to injury suffered during dystocia such as a cervical or uterine mural tear, a sequela of a postpartum problem such as retained placenta, or poor vulvar conformation and chronic windsucking. A variety of foreign material may be found in the uterine lumen including fecal matter, urine (calcium carbonate crystals), purulent exudate or mucus, tips of culture swabs, or mummified parts of fetuses.[87] After endoscopy, if there is any doubt about asepsis, it may be beneficial to eliminate contaminants by uterine lavage and intrauterine antibiotics.

Problem mares are frequently identified by their inability to mount an appropriate cellular response to contamination of the genital tract. They may accumulate fluid, experience an increased incidence of early embryonic death, or suffer chronic uterine infections. The goal in evaluating a mare's reproductive ability is to determine not only what is normal or abnormal but also the treatment and management techniques that can best take advantage of the mare's remaining reproductive potential.

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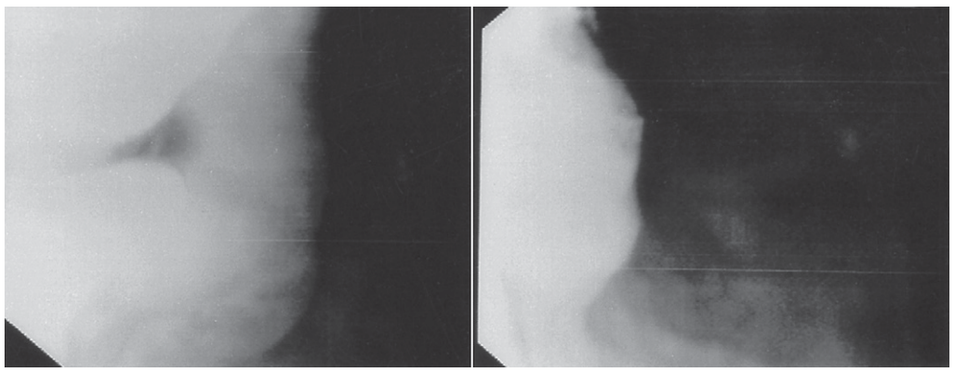

A **B**

Fig. 9-16 Laser ablation of a broad-based, multilocular cyst before **(A)** and after **(B).** The flocculent material in **B** is attributable to the cyst contents, which were released into the lumen and subsequently removed upon uterine lavage. (From Threlfall WR, Carleton C. In Traub-Dargatz J, Brown C (eds): *Equine endoscopy,* 2nd ed. St. Louis: Mosby, 1996.)

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CHAPTER 10

Control and Synchronization of the Estrous Cycle and Ovulation

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Accurate and predictable synchronization of estrus and ovulation is vital to equine breeding. The objectives of controlling the reproductive cycle of mares during the breeding season center on the ability to induce a fertile estrus and ovulation.

Synchronization of estrus has been used successfully as a management tool in the cattle industry. In cows, estrus and ovulation are simultaneously synchronized by hormonal therapies because ovulation predictably occurs shortly after the end of estrus (see Chapter 38). In contrast, synchronization of the mare's estrus and ovulation is more difficult.

The mare's estrus is relatively long, averaging 5 to 7 days; the maturation of the preovulatory follicle until ovulation takes 36 to 48 hours to complete. In addition, ovulation cannot be predicted from the first day of estrus as in cows. Actually, in the mare, behavioral signs of estrus typically cease within 2 days after ovulation; thus, the presumptive day of ovulation can only be determined retrospectively. Even though many effective methods have been developed for inducing estrus with a reasonable degree of synchronization, hormonal therapies that predictably induce estrus and ovulation are limited. In the United States, very few drugs have been approved for control of the equine estrous cycle. Because many horse breed associations are now accepting the use of frozen and shipped cooled semen in artificial insemination programs, the development of synchronization protocols that would allow a single breeding at a predetermined time remains a challenge for veterinarians and a great expectation for horse breeders.

This chapter focuses on methods that are currently available for controlling and synchronizing estrus and ovulation in mares, including induction of cyclicity in anestrous and transitional mares.

INDUCTION AND SYNCHRONIZATION OF ESTRUS

In general terms, synchronization of estrus in mares is currently accomplished in the following ways:

1. Termination of the luteal phase of the estrous cycle with prostaglandin F_2 alpha ($PGF_{2\alpha}$)
2. Lengthening the luteal phase of the estrous cycle with exogenous progestins
3. Combination of both methods

Termination of the Luteal Phase with Prostaglandin F_2 Alpha

The luteal phase of the estrous cycle can be shortened by treatment with $PGF_{2\alpha}$ and its analogues.[1] $PGF_{2\alpha}$ was shown to be luteolytic in mares in 1972 and since then has been widely used in breeding farms that require intensive management of broodmares and stallions and mares.[2] The corpus luteum is not responsive to one single injection of $PGF_{2\alpha}$ until day 5 after ovulation, although daily administration of $PGF_{2\alpha}$ for 2 or 3 days starting on day 3 after ovulation may cause luteolysis. In horse mares, 9 µg/kg of the $PGF_{2\alpha}$ free acid equivalent to 1.2 µg of dinoprost tromethamine prostaglandin (Lutalyse, the only $PGF_{2\alpha}$ approved for horses by the Food and Drug Administration [FDA]) has been shown to be luteolytic, but the manufacturer recommends the administration of 1 mg/100 lb (~22 µg/kg). Recently, a dose as low as 0.5 mg of Lutalyse per horse mare (~1.2 µg/kg) administered twice 24 hours apart caused luteolysis in 100% (21/21) of mares in the study without inducing well-known side effects (sweating, colic) associated with recommended dose.[3]

Most common indications for the use of $PGF_{2\alpha}$ in nonpregnant broodmares include but are not limited to the following: [4]

1. Induction of estrus when the previous ovulation date is known. Specific indications may include a missed breeding, diagnostic or therapeutic considerations, induction of estrus to accommodate the stallion breeding schedule, and embryo transfer. Veterinarians using palpation per rectum and transrectal ultrasonography should determine whether the mare is in diestrus or not and administer $PGF_{2\alpha}$ accordingly; if reproductive examination is not available, horse breeders may be instructed to administer a single dose of $PGF_{2\alpha}$ 5 days after the mare ceases behavioral signs of estrus. If veterinary assistance or teasing information is not available, horse breeders might be advised to administer a single dose of $PGF_{2\alpha}$ at

any given day and to repeat it in 5 days if the mare is not observed in estrus.

2. Fertility of foal heat can be decreased in mares experiencing problems during the peripartum period (dystocia, retained placenta, delayed uterine involution, etc); for these reasons or simply because of a breeding management strategy, some horse owners may choose to not breed a mare during her foal heat. Shortening the first postpartum luteal phase is then recommended and a single dose can be administered as early as 5 days after ovulation during foal heat.

3. Treatment of prolonged diestrus. This irregularity was initially thought to result from a persistent corpus luteum (CL). Prolonged diestrus is often associated with another unique phenomenon of the mare's estrous cycle, the diestrous ovulation. Prolonged diestrus is diagnosed as a diestrous period lasting more than 16 days after ovulation. A single dose of $PGF_{2\alpha}$ is administered at any time after the condition has been diagnosed via reproductive examination (palpation of a firm, tubular cervix; detection of a CL by ovarian ultrasonography) or hormonal assays (detection of >2 ng/ml of serum or plasma progesterone). Mares undergoing early embryonic death after the maternal recognition period may also experience prolonged diestrus. Regardless of the etiologies, concentrations of plasma or serum progesterone greater than 2 ng/ml in mares with spontaneously prolonged interestrous intervals indicate the presence of a functional CL that requires treatment with $PGF_{2\alpha}$. Occasionally, mares with spontaneously prolonged interestrous intervals do not have a functional CL and would not benefit from $PGF_{2\alpha}$ therapy.

4. Estrus synchronization. In this regimen, two doses of $PGF_{2\alpha}$ are administered 14 to 18 days apart. However, for close synchrony of estrus and ovulation in a group of mares, more elaborate schemes of hormonal treatment are required.

5. Induction of estrus following therapy with progesterone (or synthetic progestin) used to suppress estrus and pituitary release of gonadotropins. A single dose of $PGF_{2\alpha}$ is administered on the last day of progestin treatment to ensure that all luteal function is negated.

Other indications for administration of $PGF_{2\alpha}$ include termination of unwanted pregnancies before and after formation of endometrial cups, termination of abnormal pregnancies such as trophoblastic vesicles without an embryo proper, and therapy for mares susceptible to persistent postmating endometritis. Induction of parturition with $PGF_{2\alpha}$ is possible but not recommended because of an increased incidence of premature placental separation and decreased foal viability associated with its use.

Idiosyncrasies of Prostaglandin F_2 Alpha Use in Mares

Without a sound understanding of endocrinology and knowledge of the follicular and luteal structures present in the ovaries at the time of $PGF_{2\alpha}$ administration, some of the clinical responses observed (such as the interval from treatment to display of estrus and time of ovulation) may be confusing to veterinarians not familiar with the mares' reproductive physiology.

Prostaglandin $F_{2\alpha}$ only has a significant effect when a mature CL is present.[5] Maturity of a CL and hence its responsiveness to the action of $PGF_{2\alpha}$ may vary considerably among individual mares. Although the CL of most mares will respond to $PGF_{2\alpha}$ from the fourth day after ovulation, some will respond as early as the first day after ovulation, and others appear to be refractory.[6] Studies in cows have shown that stage of diestrus[7] and follicular status at the time of $PGF_{2\alpha}$ administration[8] have dramatic effects on the interval between $PGF_{2\alpha}$ treatment and display of behavioral estrus. Large and small luteal cells have been shown to differ in their response to luteotrophic and luteolytic agents through regulation of $PGF_{2\alpha}$ receptors.[9] The mare's CL has two distinct populations of luteal cells but it has been reported that the relative proportion of small to large luteal cells increases with the age of the CL during diestrus.[10] The effect of this phenomenon on clinical response to treatment with $PGF_{2\alpha}$ is currently unknown.

Follicular status also has an effect on the interval from treatment with $PGF_{2\alpha}$ to the onset of estrus and ovulation.[4] When a large follicle (30 to 40 mm or greater) is present at the time of $PGF_{2\alpha}$ administration, a number of different clinical responses have been demonstrated.[5] In most cases, mares come into estrus and ovulate within 6 days after $PGF_{2\alpha}$ treatment,[11] but in a few instances ovulation occurs within 24 to 72 hours and the mare shows few or no signs of estrus.[12] In the latter situation, $PGF_{2\alpha}$ may play a role in accelerating ovulation, because analogues of $PGF_{2\alpha}$ have been shown to speed the ovulatory process.[13] On occasion, the large diestrual follicles present at the time of $PGF_{2\alpha}$ treatment may be atretic. Ovulation at the subsequent estrus is the result of growth and maturation of smaller follicles following regression of the larger one. In this situation, an increased amount of time is required for the mare to display signs of estrus and to ovulate.

Spontaneous diestrual ovulations occur in approximately 5% of estrous cycles and may account for apparent failure of luteolysis following treatment with $PGF_{2\alpha}$.[4] If $PGF_{2\alpha}$ is administered shortly before spontaneous ovulation of a large (greater than 35 mm) diestrual follicle, luteolysis of the primary CL may occur, but the CL that develops following spontaneous ovulation of the diestrous follicle (and increased progesterone concentrations) may mask the effects of lysis of the primary CL and the mare may not return to estrus as anticipated.

From this discussion it is clear that the use of $PGF_{2\alpha}$ in mares may not be straightforward. Administration of $PGF_{2\alpha}$ on days 6 to 9 after ovulation will result in the onset of estrus and subsequent ovulation within 3 to 4 days and 9 to 10 days, respectively, following treatment in most mares.[5] It should be emphasized that the behavioral signs of estrus may not be observed and the interval between treatment and ovulation may range from 2 to 15 days.[14]

Appropriate adjustments in the management of individual nonpregnant mares may be required when treatment with $PGF_{2\alpha}$ is anticipated. For example, careful examination of the ovaries by palpation per rectum and

ultrasonography, noting the exact size, location, and texture of follicular structures prior to $PGF_{2\alpha}$ treatment may alert the practitioner to the need to examine the mare more frequently so that short intervals between treatment and estrus and ovulation do not result in missed breeding opportunities.

Lengthening the Luteal Phase with Exogenous Progestins

During the physiologic breeding season when mares cycle regularly, the primary indication for extending the luteal phase is synchronization of estrus and ovulation for embryo transfer and for timed insemination. The administration of progesterone (or synthetic progestin) alone or in combination with estradiol-17β suppresses estrus behavior, thus mimicking an extended luteal phase for as long as the progestins are administered.[14] Progesterone is also used alone to assist in the maintenance of pregnancy and to delay the onset of estrus (e.g., at foal heat). Because progesterone has an inhibitory effect on the release of LH from the anterior pituitary, the rationale is to artificially maintain an elevated concentration of progesterone until the corpora lutea (CL) in all treated animals have regressed. Estrus is delayed by progesterone administration until the onset is desired. Following withdrawal of progesterone, estrus and ovulation should occur at a predictable time. This method is generally successful for estrus synchronization in mares; however, progesterone, as with any other single hormone used to synchronize estrus and ovulation in mares, does not exert complete control over the physiologic events of the mare's estrous cycle. The limiting factor is the degree to which spontaneous follicular growth and ovulation are suppressed.

Treatment with progesterone in oil (50 mg/day IM) initiated before estrus prevents the behavioral signs of estrus but does not prevent ovulation, whereas a dose of 100 mg/day blocks both estrus and ovulation.[15] Neither dose is very effective if started after the first day of estrus. A dose of 200 mg/day is needed to suppress behavioral signs if a mare is in estrus at the beginning of treatment. Also, the dose required to inhibit ovulation once estrus has begun is higher than that required by mares in diestrus. Even the high endogenous concentrations of progesterone that are present during the midluteal phase of the estrous cycle are sometimes not sufficient to suppress gonadotropin release, follicular development, and ovulation. Administration of progesterone in oil (100 to 200 mg/day IM) for 7 to 10 days is followed by estrus 2 to 7 days after treatment ends. Daily injections of progesterone in oil may be painful, are not well tolerated by mares, and have the potential to cause seromas, abscesses, and fibrosis at the injection site.

In attempts to overcome the inconvenience of daily injections, other preparations and routes of administration have been tested; however, none are currently approved for use in horses and are commercially available only through veterinary compounding pharmacies. Intravaginal sponges impregnated with progesterone and synthetic progestins may have some degree of success in synchronizing estrus in mares.[16] Unfortunately, sponges have the potential to adhere to the vaginal wall and induce necrotic vaginitis. Intravaginal devices that release progesterone slowly have been investigated in Europe and Australia and hold promise for future use. Microspheres containing progesterone and progesterone plus estradiol-17β have been injected to control or synchronize the estrous cycles of mares. Some long-acting preparations of progesterone plus estradiol were available by prescription from a pharmacy in Kentucky but the FDA recently seized compounded equine drugs. The FDA understood the pharmacy was illegally manufacturing drug products from bulk ingredients without FDA approval and selling them outside a valid veterinarian-client-patient relationship.

The only progestin approved by the FDA for use in horses is altrenogest (allyl-trenbolone*). Consequently, altrenogest (Regu-Mate) is the most extensively studied synthetic progestin. Altrenogest is widely used because of the convenience of oral administration, safety, and a common belief in its efficacy. Examples of efficacy include the following:

1. A dose of 30 mg altrenogest administered orally to a group of mares for 10 to 15 days resulted in 80% of the mares being in estrus within 2 to 8 days after treatment was suspended and 79% of mares ovulating within 7 to 12 days after treatment was suspended.
2. Oral administration of altrenogest to mares at a dose of 0.044 mg/kg body weight for 15 days resulted in intervals to estrus and ovulation of 5.0 ± 2.4 days and 10.2 ± 3.6 days, respectively.

When altrenogest is fed for 15 days, most mares express estrus within 3.5 to 5 days and ovulate 9 to 11 days after treatment.[17]

Lofstedt and Patel[18] questioned the ability of altrenogest to control the equine estrous cycle. At the recommended dose and duration of treatment, altrenogest failed to suppress follicular development. Ovulation occurred in three of four mares when treatment was initiated during the first 3 days of estrus. Apparently the binding affinity of altrenogest for receptors in the hypothalamus is 60% that of progesterone. Also, LH is only slightly or not at all suppressed by altrenogest.[16] Of the four mares that were treated during estrus in one study, the mean duration of estrus was 4.5 days. Nine of the 12 mares treated with altrenogest ovulated and formed CL during treatment. In four of the mares, the CL persisted between 2 and 10 days beyond the 15-day altrenogest treatment. To ensure closer synchrony, $PGF_{2\alpha}$ should be administered at the end of the progestin therapy. Altrenogest or progesterone in oil given for 9 days in addition to an injection of $PGF_{2\alpha}$ on day 9 or 10 of treatment with progestin has been shown to be an effective method of synchronizing estrus. Oral administration of altrenogest for 8 to 12 days with an injection of $PGF_{2\alpha}$ on the last day of altrenogest treatment will result in most mares exhibiting estrus in 2 to 5 days after withdrawal. If any large follicles exist at the end of treatment, they can ovulate without the mare exhibiting signs

*ReguMate, Hoechst-Roussel Agri-Vet Co, Somerville, NJ.

of estrus, and these ovulations may be missed. However, not all mares with preovulatory follicles present at the end of treatment ovulate within 3 to 4 days. In one study, 4 of 12 preovulatory follicles remained until ovulation occurred 6 to 9 days later.[17] Examination of the ovaries with transrectal ultrasonography at the end of the treatment period and administration of human chorionic gonadotropin (hCG) when a preovulatory follicle has reached 35 mm in diameter will help alleviate this problem and provide tighter synchrony of ovulation. During routine breeding management of the majority of mares at breeding operations, there are few indications for use of progestins to control the reproductive cycle.

INDUCTION AND SYNCHRONIZATION OF OVULATION

Induction of Ovulation with Human Chorionic Gonadotropin

In the mare, when normal estrous cycles have been established it is probable that high-frequency pulses of gonadotropin-releasing hormone (GnRH) from the hypothalamus are responsible for eliciting a preovulatory increase in luteinizing hormone (LH).[19] This increase appears to be directly associated with final maturation of preovulatory follicles and initiation of ovulation. The process of ovulation involves a complex interaction between LH and cyclic adenosine monophosphate, prostaglandins, and proteolytic enzymes.[20] Follicles increase rapidly in size (about 5 mm in diameter per day) before ovulation. In 400- to 500-kg mares, preovulatory follicles average 44 mm in diameter.[1]

The ability to accurately control ovulation in mares and synchronize ovulation with mating has several advantages. The use of an ovulation induction agent effectively minimizes the number of matings required per estrous cycle, thereby conserving stallion power.[21] Administration of a drug to induce ovulation in mares shipped to the stallion for breeding ensures a minimum of transportation during each breeding cycle and reduces the chance of stress-induced anovulation. Mares prone to uterine infection as a result of breeding also benefit from one cover or insemination per estrous cycle.[22]

Human chorionic gonadotropin (hCG) is a hormone that induces ovulation in most domestic animals and humans. The hCG hormone is extracted from the urine of pregnant women and provided as a lyophilized product that must be reconstituted shortly before use because its stability in solution is relatively short (2 to 3 weeks). Although hCG has been used to induce ovulations in mares since 1939,[23] it is not approved by the FDA for use in horses. The hCG is an LH-like hormone and thus has been used extensively to induce or hasten ovulation at a predictable time during estrus.[24] Before the advent of ultrasonography, several studies have indicated that administration of hCG on the second day of estrus can shorten the mean length of estrus by 1 to 3 days.[24,25] Administration of a single dose of 1500 to 3300 IU results in ovulation within 24 to 48 hours and shortens estrus in treated animals compared with untreated control animals.[1] A dose of 2500 IU IV is typically administered when a follicle 35 mm or greater in diameter is detected by palpation per rectum or ultrasonography in an estrual mare and a breeding appointment has been arranged. Mares with two presumptive preovulatory follicles do not need a second injection of hCG during the same cycle. The stage of follicular development at which hCG is administered is very important. Constant monitoring (every 24 or 48 hours) of follicular development throughout estrus by transrectal ultrasonography is essential for a predictable response. Generally, poor responses have been noted when hCG was given before follicles reached 30 mm in diameter or when a large follicle destined to atresia is present in the ovaries. Appropriately sized follicles (35 mm in diameter) are detected on the second or third day of estrus, and ovulation usually occurs before 48 hours after injection. In one trial, 83 of 89 of mares ovulated within 48 hours after injection.[26] Recent reports have shown that efficacy of hCG to cause ovulation was not affected by follicle size, as long as the minimum follicle diameter of 30 mm had been reached. Ovulation rates for follicles measuring 30 to 34 mm, 35 to 39 mm, and over 40 mm were not significantly different.[27] For more accurate control and synchronization of ovulation in a group of mares (e.g., embryo donors and recipients), hCG can be included as part of an estrous synchronization regimen (see later discussion).

Disadvantages of hCG administration are inconsistencies in response between and within mares and antibody formation, which may or may not cause refractoriness and impair fertility.[28] Refractoriness and decreased fertility seems to be associated with elevated doses of hCG (>5000 IU) administered intramuscularly. Although the product insert recommends the intramuscular route, equine reproductive physiologists recommend the intravenous route because, in addition to being a safe route, it is expected to minimize antibody formation. As most hCG products in the United States are sold as 10,000 IU lyophilized vials that must be reconstituted into 10 ml of distilled water before use, it is convenient to administer 2000 to 3300 IU per animal (3 to 5 doses per vial).

Induction of Ovulation with the GnRH Analogue Deslorelin

Gonadotropin-releasing hormone (GnRH), a 10-amino-acid neuropeptide, has recently been shown to induce ovulation in mares.[29] However, treatment with GnRH or its analogues administered as a single injection[25,30–32] or multiple injections[33–35] during estrus in cyclic mares has resulted in variable efficacy. Continuous or multiple treatments were more efficacious than a single treatment.

The nonapeptide deslorelin is a potent (relative potency of 114) analogue of the natural decapeptide GnRH. Recently, studies using deslorelin (6-D-tryptophan-9-[(*N*-ethyl-L-prolinamide]-10-desglycinamide) delivered by a novel release system (Ovuplant)* have shown the effects of deslorelin administration on ovulation.[36,37] Ovuplant is a biocompatible sustained-

*Ovuplant, Fort Dodge Animal Health, Fort Dodge, IA.

release subcutaneous implant and its registration by the FDA was achieved in June 1998 for sales in the United States. Deslorelin (Ovuplant) has become the first hormone approved for inducing ovulation in mares in the United States. Studies in Europe during the 1990 breeding season demonstrated that short-term implants containing deslorelin induced ovulation in cyclic mares comparably to 3000 and 5000 IU hCG.[36] Two dose-response studies, one in Australia[29] and one in the United States,[37] demonstrated that a dose of 2.2 mg deslorelin given as a deslorelin short-term implant was appropriate for reliable acceleration and induction of ovulation. Most recently, deslorelin has been shown to effectively induce ovulation in mares without diminished activity over three consecutive estrous cycles.[38] Recent field trials with deslorelin, delivered by a subcutaneous slow-release biocompatible implant, revealed an ovulation rate (within 48 hours of administration) of 88.2% for deslorelin-treated mares, compared with 37.7% in placebo-treated mares.[39] Ovuplant is currently available in the United States only in implants containing 2.1 mg of deslorelin already preloaded in an implanter designed for subcutaneous administration. Its efficacy in inducing ovulation in mares is comparable to that of hCG; however, the deslorelin implant is relatively more expensive than hCG (hCG costs approximately one sixth of Ovuplant price).

Ovuplant was well received in the market because in addition to being an FDA-approved drug, it was an attractive alternative to mares presumed to be refractory to hCG administration. Deslorelin, as opposed to hCG, does not induce antibody formation. During the second breeding season after Ovuplant was in the U.S. market, anecdotal reports discussed the increased number of mares that would have prolonged interestrous intervals had they not become pregnant in the cycle Ovuplant was used. In 2000 and 2001, Louisiana and Colorado researchers, respectively, reported that deslorelin could indeed induce prolonged interovulatory intervals in cycling mares; this effect was probably caused by a prolonged decrease of FSH plasma concentrations during the luteal phase that followed deslorelin-induced ovulations.[40,41] Soon thereafter, it was recommended to have the implant removed once ovulation was confirmed by reproductive examination to avoid prolonged effects of deslorelin.[42] Because it was relatively difficult to find the 3-mm pellet in the area in which it was applied, veterinarians begun to insert Ovuplant in the vaginal mucosa, just short from the vulvar mucocutaneous junction to facilitate the visualization of the pellet for its removal. Administration of $PGF_{2\alpha}$ to short cycle a mare in the diestrus following administration of Ovuplant is not recommended, especially if the bioimplant has not been removed. The use of Ovuplant is now, in addition to its relatively higher cost, associated with increased labor.

"Ideal Method" of Synchronizing Estrus and Ovulation

The variation in duration of estrus makes synchronization of ovulation difficult. Precise control over ovulation is necessary for success in embryo transfer programs. None of the methods described here is entirely effective for precise control of ovulation. When the above methods are used in combination, synchronization of ovulation is improved. In randomly cycling mares, synchronization of ovulation is improved when mares are treated with a combination of progesterone and estradiol rather than progesterone alone. Combining these hormones exerts a more profound negative feedback on LH and FSH release than progesterone alone, resulting in more uniform inhibition of follicular development. When exogenous progesterone and estradiol therapy is discontinued, a more uniform follicle population begins to develop in the ovaries, resulting in a more synchronous ovulation. Mares treated with this drug combination do not experience decreased fertility.

The protocol, originally developed by Loy and coworkers,[43] involves intramuscular injections of progesterone (150 mg) combined with estradiol-17β (10 mg) in oil for 10 consecutive days with a single injection of $PGF_{2\alpha}$ administered on the tenth day of treatment. After the combined steroid treatment regimen is discontinued, administration of hCG (2500 IU) IV or IM when a 35-mm follicle is detected will further improve ovulation synchrony. No commercial preparations of estradiol-17β are approved for use in horses. Estradiol cypionate and estradiol benzoate as substitutes for estradiol-17β in this synchronization scheme have not been investigated. It is likely that the prolonged action of these formulations would make their effects less predictable and therefore less desirable.[44]

POSTPARTUM MARES

Administration of ecbolic agents (e.g., oxytocin and $PGF_{2\alpha}$) has been advocated to enhance uterine involution and thereby improve fertility during the early postpartum period, but results of studies have been extremely varied and inconclusive.

Two methods are currently employed to postpone breeding during the postpartum period until normal pregnancy rates can be achieved. These methods delay the onset of foal heat or shorten the interval to the second postpartum estrus. Pregnancy rates achieved by breeding during foal heat appear to be higher in mares in which estrus is delayed by hormonal therapy.

In the first method, altrenogest is given orally (0.044 mg/kg/day) for 8 to 15 days after foaling,[45] followed by $PGF_{2\alpha}$ on the last day of treatment. Prostaglandin is given because progestin therapy alone may not prevent ovulation even though estrus is suppressed. If ovulation has occurred during altrenogest treatment, the resulting CL will continue to produce progesterone despite withdrawal of the exogenous progestin. Treatment with $PGF_{2\alpha}$ ensures that progesterone concentration will decrease and permit the mare to return to estrus, usually within 2 to 7 days. A combination of progesterone and estradiol-17β as described earlier also delays the onset of foal heat and ovulation.

Delay of foal heat with hormone therapy until after day 10 postpartum allows more complete uterine involution to occur, as evidenced by a greater proliferation of endometrial glands and increased endometrial gland density and ciliation of luminal epithelium.[46] Delaying

foal heat with progesterone likely improves the uterine environment and endometrial secretions necessary to sustain conceptus development.

One objection to the use of progestins for several days beginning at the time of foaling is that treatment delays the onset of foal heat and foaling intervals are not significantly reduced. If treatment of postpartum mares for 2 or 3 days after foaling would delay ovulation until just after day 10 post partum, hormone treatment might offer the best method for increasing pregnancy rates without significantly extending the foaling interval. There is also concern that treatment of mares with progestins during the early postpartum period might predispose to development of postpartum endometritis. Controlled studies, however, have not proved this premise.

Administration of $PGF_{2\alpha}$ 6 or 7 days after the first postpartum ovulation hastens the onset of the second postpartum estrus, which normally occurs approximately 30 days post partum.[5] Although this management technique is expected to increase the pregnancy rate at the first postpartum breeding, such is not always the case. The interval from foaling to first breeding will also be about 2 weeks longer than that associated with breeding during foal heat. About 1 week is saved by breeding during foal heat compared with waiting and breeding during the $PGF_{2\alpha}$-induced second postpartum estrus.

The ideal method to ensure optimal pregnancy rates at the first breeding regardless of whether it occurs during foal heat or during the subsequent estrus is to monitor postpartum mares by transrectal ultrasonography for ovulation and fluid accumulation within the uterus. Mares can be bred during foal heat if they do not ovulate before day 10 post partum and if normal uterine ovulation occurs; little or no fluid should be present when the mare begins its foal heat. Normal per cycle pregnancy rates can be expected under these conditions for mares bred at foal heat. If ovulation is expected to occur prior to day 10 post partum or if a significant amount of fluid is present within the uterus, the mare should be treated with $PGF_{2\alpha}$ 1 week after ovulation and bred instead during the induced estrus.

HORMONAL MANAGEMENT OF TRANSITIONAL ESTRUS

Since the 1970s, attempts have been made to hasten ovulation during the transitional season with progesterone or combinations of progesterone and estradiol. Although the mechanism of action is not completely understood, it is speculated that progesterone may act by causing a rebound release of LH following withdrawal of treatment.[20] The ability of progesterone to synchronize the first ovulation has been well documented, but the ability of progesterone to stimulate follicular development and hasten the first ovulation during the transitional season is debatable.[47] Administration of progesterone to mares that are on the verge of the first ovulation of the year may block estrus and synchronize the first ovulation, but administration of progesterone to mares earlier during the transitional season may not hasten the first ovulation. Altrenogest has been used frequently in attempts to hasten ovulation in transitional mares. Altrenogest is administered at 0.044 mg/kg/day for 15 days, and it is recommended that therapy not be initiated until the largest follicle exceeds 20 mm in diameter.

A number of preparations of progesterone and estrogen are currently in use but have to be prescribed by a veterinarian and prepared by a compounding pharmacy. Injectable progesterone (150 mg) and estradiol-17β (10 mg) administered daily for 10 to 15 days have been used to hasten ovulation in transitional mares. The addition of estradiol to the preparation results in more profound suppression of follicular development compared with progesterone alone. This therapy is frequently combined with a dose of $PGF_{2\alpha}$ on the last day of hormone administration to remove any luteal tissue that might be present in mares that have had a previously undetected ovulation.

Administration of a single dose of GnRH to anovulatory mares will result in a transient increase in LH and follicle-stimulating hormone release from the pituitary.[48] Thus, the pituitary gland is able to respond to exogenous administration of GnRH during the anovulatory season. A number of studies have demonstrated that administration of GnRH stimulates follicular development and ovulation in mares.[48] Several treatment regimens and GnRH analogues have been used to stimulate follicle development and ovulation during the anovulatory or transitional period in mares.[35] Early trials provided frequent (hourly) pulses of GnRH to stimulate gonadotropin release. Although pulsatile administration of GnRH may improve the response to therapy compared with continuous administration, a number of studies have shown that continuous administration of GnRH or an analogue is effective in stimulating follicle development and ovulation in treated mares. Administration of relatively potent GnRH analogues twice daily has also been effective in hastening the first ovulation in anovulatory or transitional mares.

Administration of GnRH or its analogues to mares during the anovulatory period reduces follicular response and ovulation in some mares. The response to GnRH therapy appears to be more predictable in mares that have entered the transitional period and in which some follicular development is present. Mares in deep seasonal anestrus require longer time to respond to exogenous GnRH, and these mares are more likely to return to an anovulatory state after an induced ovulation. Some mares induced to ovulate with GnRH during the deep anestrus period failed to form a CL. When ovulation was induced with GnRH during deep anestrus (follicles less than 15 mm in diameter), the pregnancy rate at day 11 after ovulation was 64%; however, the rate of pregnancy loss by day 40 was also 64%.[50] These losses appeared to be related to low serum progesterone concentrations and it appears that the CL formed after induced ovulation in mares during deep seasonal anestrus are not functional. When ovulation was induced in transitional mares with active ovaries, there was not an increase in the rate of embryonic loss. Therefore, the use of GnRH to induce follicular development and ovulation appears to have most benefit in mares that have entered the transitional phase and that have detectable follicular development (greater than 20 mm in diameter) at the start of treatment.

A variety of delivery systems have been used for the administration of GnRH.[51] These include the use of continuous delivery from an osmotic minipump implanted subcutaneously, which delivers its content over a number of days. Multiple injections (ranging from hourly to twice daily) have been used successfully, but this type of treatment is too intensive for practical application. Pulsatile delivery systems are available but the equipment is expensive and not used routinely. The most promising delivery system for the administration of GnRH to transitional mares is the use of controlled release implants that deliver a constant amount of the drug for up to a month.

Mares ovulated approximately 18 days after initiation of GnRH treatment during the transitional season. Fertility of mares induced to ovulate during the transitional season with GnRH appears comparable to that of control mares. Pregnancy rates for induced ovulations are high (80% in one study), although more work is required to characterize fertility of mares treated with GnRH during transition. Currently, the only options available for administration of GnRH to anestrous or transitional mares are by injection or an osmotic minipump. The use of multiple implants of deslorelin has been shown to induce ovulation in the early transitional mare; however, this regimen is not recommended because prolonged interovulatory intervals could result from the use of multiple Ovuplants in a short period of time.

Human chorionic gonadotropin may also be effective during the late transitional season to hasten the first ovulation. Administration of 3300 IU hCG to transitional mares with a follicle greater than 40 mm in diameter that have been in estrus for more than 3 days accelerated the first ovulation by approximately 1 week compared with control mares. The function of the CL that developed after ovulation was induced with hCG appeared normal. Because normally several (up to four) follicular waves occur during the transitional season, it may be difficult to determine whether a large follicle present on the ovary of a mare is growing or regressing. If the follicle is undergoing atresia, hCG will probably not induce ovulation. Therefore, it is important that follicular status be known prior to the use of hCG. This may require multiple examinations using ultrasonography to determine that the follicle is increasing in size and softening in texture.

In summary, the use of artificially prolonged photoperiods remains the most effective means available to hasten the onset of the ovulatory season in mares. The use of progesterone or progesterone and estradiol combinations during the late transitional season may synchronize the first ovulation, but it is uncertain whether this therapy will hasten ovulation. Administration of GnRH or its analogues over a 2- to 3-week period is effective in inducing ovulation in anovulatory mares. The response to GnRH therapy depends on the size of follicles present at the start of treatment. Mares with active ovaries (follicles greater than 20 mm in diameter) appear to respond more reliably than mares with smaller follicles. hCG can hasten the first ovulation of the year during the late transitional season if administered during the growing phase of follicle development and a well-developed uterine edema is present.

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CHAPTER 11

Pregnancy Evaluation in the Mare

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Effective management of broodmares requires that their pregnancy status be known so that proper managerial steps can be taken to ensure optimal care. At 14 to 18 days after ovulation, a mare that has been bred should be carefully examined for pregnancy; if the mare is not pregnant, steps can be taken to have her bred prior to the next ovulation. Early detection of twins is imperative for a successful reduction to a single pregnancy by manual crushing of one conceptus. If performed after 30 days of gestation, the procedure is less likely to result in a single, viable fetus. Ideally, mares that have been pronounced in foal should be teased three to four times per week and re-examined at approximately 30, 42, 60, and 120 days and again in the fall to monitor their reproductive status. If pregnancy loss has occurred, they can be rebred expeditiously if no obvious cause is detected that requires treatment. Broodmares that are not pregnant at the end of the breeding season should undergo a breeding soundness examination. Therapy, if indicated, can then be instituted and the mare's reproductive status be re-evaluated prior to the onset of the next breeding season.

Knowledge that a mare is pregnant allows one to make the decision to vaccinate for protection against equine herpesvirus I (rhinopneumonitis) periodically throughout gestation (3, 5, 7, and 9 months) and to administer other vaccines as appropriate for the geographic location 1 month prior to the mare's anticipated due date. Use of anthelmintics should be avoided the first 60 days of gestation during organogenesis, as well as during the last 30 days of gestation, when any abortion/stillbirth that might occur may be attributed to their use. Otherwise, appropriate anthelmintics approved for use during pregnancy (consult the product label and package insert carefully) should be administered periodically throughout gestation. The frequency of anthelmintic administration will be determined by the environment and animal density, but may be as often as every 6 to 8 weeks or even on a daily basis. During the last trimester, pregnant mares may need to be separated from open mares so that additional feed can be provided as necessary to maintain optimal development of the fetus. The pregnant mare herd should be isolated from any transient population to prevent exposure to infectious diseases. Mares greater than 300 days pregnant should be closely observed for signs of mammary gland development and impending parturition.

Several techniques are available to determine the presence of an embryo or fetus and the status of pregnancy. Some techniques are used to directly evaluate the conceptus or fetus; other techniques are used to indirectly assess the conceptus by evaluating changes in the mare's behavior, hormones, or genital tract that are due to pregnancy. The procedure used will be based on factors such as stage of gestation, temperament, size, reproductive or medical history of the mare, examination facilities, equipment available, intensity of management, financial constraints of the owner, value of the mare, and experience of the veterinarian.

BEHAVIOR

Although the absence of estrus may suggest the pregnancy status of some species, it is not a reliable indicator in the mare. Ten percent of pregnant mares may show estrus during pregnancy. Estrus during pregnancy typically occurs around 18 to 21 days after ovulation. Mares that experience pregnancy loss between 40 to 120 days of gestation typically do not come back into estrus until after the endometrial cups have involuted. Nonpregnant mares would likely fail to display estrus during the winter anestrous season.

PALPATION PER RECTUM

Characteristic changes occur in the mare's genital tract during pregnancy. Some changes are due to the response of the genital organs to hormone stimulation. Changes in dimensions of the uterus and the position of the genital tract within the pelvic canal are caused by an increase in size of the growing conceptus and increase in fetal fluid volume. The large size and tolerant nature of most broodmares permit evaluation of changes in the internal genitalia by palpation per rectum.[1] Careful, systematic palpation of the internal genital tract per rectum requires generous lubrication, gentle removal of feces, and adequate restraint of the mare. To prevent damage to the rectal mucosa, the tail should be wrapped or care taken to avoid inadvertently dragging tail hairs into the rectum during the examination. A water-soluble methylcellulose lubricant is recommended because it is not irritating to the rectal mucosa. The lubricant should be rinsed or wiped from the perineum after the examination to prevent chapping of the perineum. The rectum must

be emptied of feces so that the genital organs can be felt. Mares can be palpated per rectum while being held in hand, standing in a stall doorway or in stocks. A nose twitch may be required to more safely restrain restless or difficult mares. As with any exogenous medications, administration of tranquilizers should be avoided during pregnancy. Nonetheless, if the results of the examination are critical for making management decisions and there is risk of injury to the mare or examiner, chemical restraint of the mare may be necessary. Adequate relaxation may be achieved using acepromazine (0.05 mg/kg IV) alone. Xylazine (0.3–0.5 mg/kg IV) combined with acepromazine (0.05 mg/kg IV) has been used clinically to help nervous mares tolerate palpation per rectum. Although xylazine has little effect on fetal heart rate, it causes contraction of the uterus, increases uterine tone, and may induce premature parturition. Therefore, xylazine should be used with caution during the last trimester of pregnancy.

Cervix

As early as 16 to 18 days after ovulation, the cervix of the pregnant mare becomes tightly closed, firm, slender, and elongated. The portio vaginalis assumes a pointed shape. In multiparous mares, the cervix may not assume this character until 21 days after ovulation. These changes are not only due to elevated blood concentrations of progesterone but also due to "embryonic" factors not yet identified but thought to be associated with pregnancy. The cervix of the normal pregnant mare may remain closed until the mare goes into first stage labor, or it may soften and relax during the month prior to parturition.

Uterus

Careful evaluation of the uterine horns between 12 and 25 days of gestation will reveal a marked and gradual increase in uterine wall thickness. The endometrial folds are no longer palpable as folds of tissue rippling through one's fingertips. Instead the uterus becomes tubular, smooth, and firm with tone. This tone is caused by progesterone and embryonic factors. Distinct bends at the base of the uterine horns may be present at 20 to 22 days of gestation. The conceptus becomes positioned at the base of one of the uterine horns near the junction of the uterine body. Both the nongravid and gravid uterine horns on each side of the conceptus maintain a firm, tubular character as the conceptus increases in size. The uterine wall thins over the developing conceptus, which maintains a buoyant, vibrant quality throughout gestation. The uterine horns and body gradually lose the tubular tone after 48 to 55 days.

The conceptus develops in a recognizable pattern of size and shape, allowing an estimation of age based on its palpable characteristics. In maiden and barren mares, a careful, experienced clinician may be able to feel the embryonic vesicle ventrally at the base of one uterine horn at 17 to 25 days, producing a bulge 3.5 cm in diameter. At 30 days of gestation, both uterine horns will be small with prominent tone and the conceptus can be felt as a ventral bulge 4 cm in diameter and positioned at the base of the gravid uterine horn. The uterine wall becomes thinner over the expanding conceptus. At 42 to 45 days, the conceptus occupies approximately half of the gravid uterine horn and is 5 to 7 cm in diameter. The enlargement of the conceptus begins to involve the uterine body by 48 to 50 days and is 6 to 8 cm in diameter and 8 to 10 cm long. At 60 days of gestation, nearly the entire gravid horn and half of the uterine body are filled with conceptus, but the nongravid horn remains small. The 60-day conceptus is 8 to 10 cm in diameter and 12 to 15 cm in length. After 85 days, the turgidity of the conceptus decreases such that the fetus becomes palpable. At 90 days of gestation, the conceptus fills the entire uterus and the cranial portion of the uterus may extend over the brim of the pelvis into the abdominal cavity. After 100 to 120 days, the gravid uterus will be positioned cranial to the pelvic brim in the abdominal cavity. The ovaries will be positioned toward the midline, cranial and ventral to their normal nonpregnant position because of the ventral traction the enlarging uterus exerts on the broad ligament.

Expansion of the uterus is not a gradual filling process but rather alternately proceeds and recedes, apparently in response to transient segmental constrictions of the uterus. These constrictions seem to cause a reallocation of allantoic fluid. Reclosure of the uterine horns first occurs after day 106. Although the placental membranes interdigitate with the endometrium of the uterine horns, little fetal fluid remains in the uterine horns. At 150 days, palpation of the fetus is still possible. Between 5 to 7 months, the uterus is positioned ventrally in the abdomen and it is difficult to thoroughly evaluate the conceptus by palpation per rectum. From 6 to 7 months the horns are approximately perpendicular to the dorsal cranial aspect of the uterine body. As the conceptus continues to grow, it expands in a dorsal direction so that by 11 months the mesometrial surface of the uterine horns move closer to the dorsal surface of the uterine body and the fetus is within reach of the examiner when palpating per rectum.

Normally the fetus is active after 40 days and mobile after 70 days of gestation.[2] Fetal activity or movement of the head, mouth, and limbs and fetal mobility occur throughout the entire fetal stage of gestation. If one fails to immediately detect fetal movement during palpation per rectum in a later stage of pregnancy, it is advisable to be patient in assessing the presence of fetal activity. Several minutes may be necessary for a movement to be noted.

During early gestation (2 to 5 months) the presentation of the fetus changes owing to the fetal mobility. As fetal mobility decreases after 4 months of gestation the fetus is more likely to be in a cranial presentation. During the seventh to eighth month the hind limbs of the fetus enter the uterine horn containing the umbilical cord, and although the limbs remain active throughout gestation, they do not retract from this horn. Distinct parts of the fetus may not be discernible, and thus it is difficult to accurately assess fetal presentation solely by palpation per rectum. Fortuitous visualization of the orbit during an ultrasound examination per rectum or location of the

heart and stomach transabdominally with ultrasonography will permit a more accurate assessment.

Ovaries

The ovaries are active during early gestation. The primary corpus luteum forms from the follicle that ovulated the oocyte of the pregnancy and persists for 160 to 180 days. Numerous medium and large follicles are present on the ovaries between 40 and 60 days. Secondary corpora lutea form from ovulation and luteinization of these follicles. After 180 to 200 days, there is little ovarian activity and evaluation of the ovaries by palpation per rectum after 120 days is difficult because the ovaries are pulled ventrally under the broad ligament because of the weight of the enlarging gravid uterus.

VAGINAL SPECULUM EXAMINATION

Reproductive status can be ascertained without invading the internal genital tract by palpation and ultrasonography per rectum as well as by transabdominal ultrasonography. Nonetheless, reproductive status can be ascertained by evaluation of the cervix of the mare by direct visualization through an illuminated speculum. Speculum examination requires the mare to be restrained as for palpation per rectum. The tail should be wrapped to prevent hairs from entering the vaginal canal. The perineum should be washed with povidone-iodine scrub or mild soap, thoroughly rinsed, and dried. A sterile speculum, lubricated with sterile, water-soluble lubricant, is gently passed through the vulvar lips, vestibule, vestibular ring, and caudal vagina. An external source of light allows one to visualize the cranial vagina and the portio vaginalis of the cervix. It is not possible to prevent air from passing through the speculum, which may allow the introduction of microorganisms or induce straining by the mare. Iatrogenic pneumovagina is particularly a problem in thin mares, older or swaybacked mares, and mares with an incompetent vestibulovaginal ring. Manipulation (stimulation) of the cervix, particularly in late gestation, should be avoided because it may result in prostaglandin release and premature parturition.

The appearance of the cervix is directly related to the hormone milieu. During pregnancy, the cervix will be tightly closed by the influence of progestogens and unidentified substances produced by the conceptus. The portio vaginalis is yellow-gray, dull, dry, sticky, and in a central position in the cranial vagina. During the last month of pregnancy, the normal cervix may be soft or relaxed, so great care must be taken not to initiate an iatrogenic ascending infection.

ULTRASONOGRAPHY

The use of ultrasonography per rectum requires the mare to be handled and restrained as one would for examination by palpation per rectum. Thorough palpation of the genital tract must be performed before an ultrasound scan is made. A real time linear 5 to 10 MHz probe is used for reproductive evaluation per rectum, which produces a rectangular cross section image of the structure scanned. The resolution of a 2.5 to 3.5 MHz probe is less than that of higher resolution transducers, but its increased depth of penetration makes it useful for transabdominal scanning of late gestation mares. The use of the ultrasound transabdominally requires that the mare's abdominal hair be clipped and the skin generously lubricated.

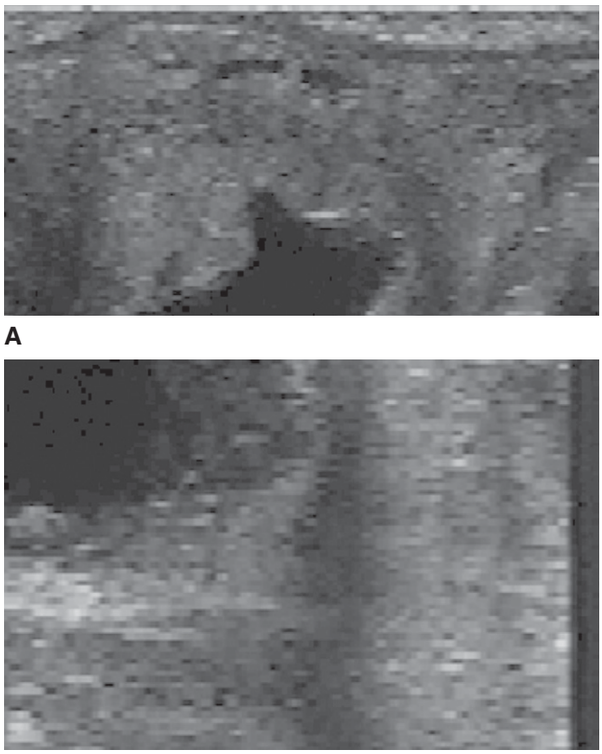
The diagnosis of pregnancy in mares using ultrasonography was first reported in 1980.[3] This technology has increased veterinarians' ability to estimate gestational age and evaluate equine pregnancy considerably. Management of twins has improved and it is recommended that mares be examined for presence of twins by ultrasonography per rectum between 14 and 19 days. Success is good for reduction of a twin pregnancy to a singleton if the manual reduction procedure is performed before 30 days of gestation (Fig. 11-1, *A* and *B*). Comprehensive reviews of the changes in the developing embryo as seen by ultrasonography per rectum are available.[4] Characteristics of the late term fetus and its uteroplacental unit obtained by ultrasonography per rectum and transabdominally allow clinicians to evaluate the status of late pregnancies.

Early Gestation

Although most equine embryos enter the uterus as a morula or early blastocyst 6 days after ovulation, the conceptus is not large enough to be seen by ultrasonography per rectum until day 9 or 10, when it appears as a round (4 mm) nonechogenic structure in the uterine lumen (Fig. 11-2). The conceptus migrates throughout the uterine lumen until day 16, at which point it lodges at the base of one uterine horn. At day 17 or 18, the conceptus has a characteristic triangular shape resembling a guitar pick. The embryo proper can first be seen in the ventral portion of the yolk sac at day 21. The allantois develops from an outpouching of the hindgut and can be seen at day 21 or 22. A comparative increase in size of the developing allantois relative to the regressing yolk sac allows estimation of the age of the embryo. At day 24 or 25, a heartbeat can be seen in the embryo proper (Fig. 11-3). At day 28 to 30, the dorsal regressing yolk sac is nearly equal in size to the developing allantois (Fig. 11-4). This relative change in the fluid cavities continues until day 40, when the allantois occupies the entire fluid cavity surrounding the amniotic vesicle (Fig. 11-5). At this time it appears that the embryo is suspended from the dorsal wall of the uterine horn by its umbilicus. Over the next few days the umbilical cord lengthens and the fetus will come to rest on its dorsum on the ventral wall of the gravid uterine horn (Fig. 11-6). After 40 to 45 days, fetal movement may be detected.

The conceptus grows at a steady rate of 3.4 mm per day from days 11 to 16. A plateau of growth occurs between days 18 to 26, after which growth continues at approximately 1.8 mm per day until day 50. The growth rates of different horse breeds are similar for the first 28 days of gestation, but thereafter, the diameter of the embryonic vesicle of draft breeds will be 1 to 4 mm larger than light horse breeds.

Absence of a heartbeat after 25 days is a reliable sign of fetal death. Impending embryonic death may be



A

B

Fig. 11-1 **A,** Twenty-day-old adjacent twins located at the base of one uterine horn. **B,** One embryo remains after the collapse of its twin by manual crushing.

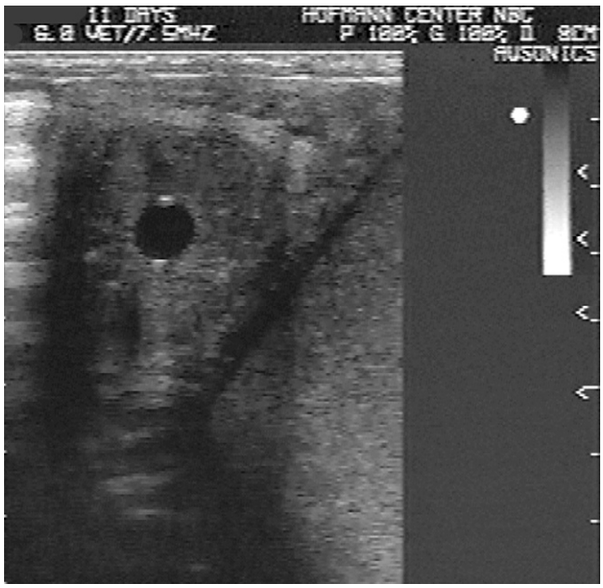


Fig. 11-2 An 11-day-old conceptus seen as a nonechogenic circle bordered by two white lines (specular reflection).

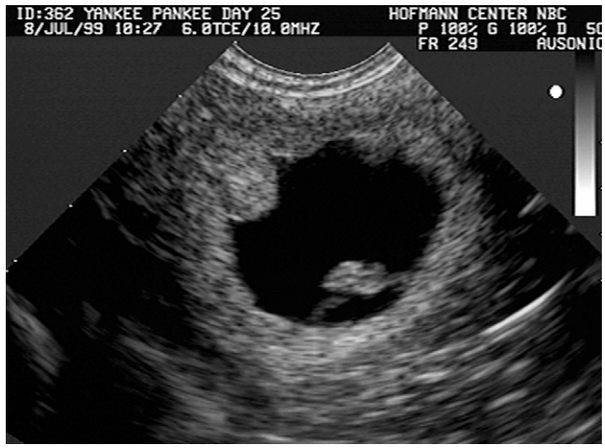


Fig. 11-3 The ventrally positioned allantois develops as a outpouching of the hindgut. The relative size of the dorsally positioned yolk sac continues to decrease as the allantois increases in size. A heartbeat would be obvious in a 25-day conceptus.

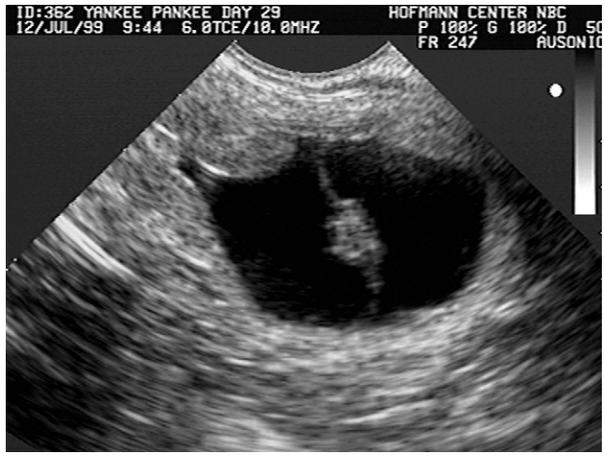


Fig. 11-4 At 28 to 30 days, the conceptus appears to contain two fluid compartments of equal size (yolk sac dorsally on left, allantoic cavity ventrally on right).

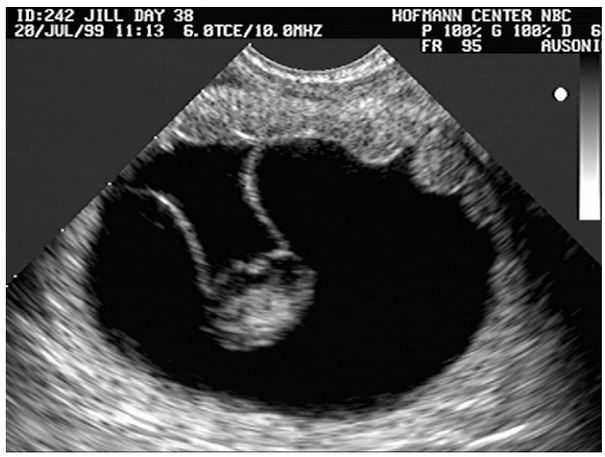


Fig. 11-5 A 38-day conceptus. The yolk sac is quite small relative to the enlarging allantois.

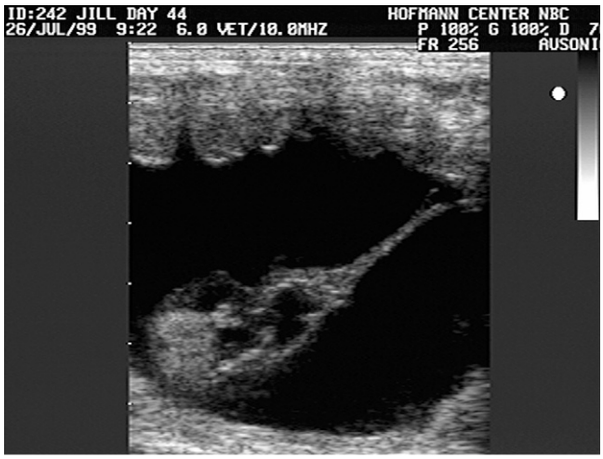


Fig. 11-6 A 44-day conceptus. The fetus has dropped to the ventral portion of the conceptus, and its umbilicus is attached dorsally to the allantois.

predicted by several characteristics seen ultrasonographically. Any increase in echogenicity of the normally nonechogenic early conceptus indicates an increase in the cellular content of the embryonic fluid. Inadequate size for the stage of gestation may indicate impending embryonic death. Subsequent to reduction of a twin pregnancy to a surviving singleton, orientation of the membranes within the conceptus may be atypical with regard to positioning of the developing allantois in relation to the regressing yolk sac. When embryonic shape is abnormal or the outline of the conceptus appears irregular such that endometrial folds can be readily seen, the abnormality is thought to be due to a decrease in surface tension of the conceptus caused by a decrease in embryonic fluid.

Fetal Sexing

Ultrasonography can be used to determine the sex of an equine fetus by imaging the position of the genital tubercle between 55 and 90 days of gestation.[5] The precursor to the clitoris in the female and the penis in the male, the genital tubercle appears as a hyperechoic bilobed structure located on the ventral midline of the fetus between the umbilicus and the tail. Each lobe of the tubercle is oval and elongated. Although the genital tubercle is first visible on examination by ultrasonography per rectum between 40 and 54 days, prior to days 55 to 59 it is in an undifferentiated position between the hind legs that does not allow sex determination. After 55 to 59 days the genital tubercle is positioned just caudal to the umbilical cord in a male and just cranial to the tail in a female. Examinations per rectum are most easily and accurately performed between 59 and 68 days. As the gravid uterus increases in size it becomes more difficult to reach the genital positions per rectum. Between 3 and 5 months of gestation, the external genitalia of the fetus (clitoris and mammary glands of the female, prepuce and penis of the male) may be imaged by ultrasound examinations per rectum (using a 2.5 to 3.5 MHz transducer) if the fetus is in caudal presentation. After 100 to 120 days of gestation the external genitalia may be imaged by transabdominal ultrasonography.

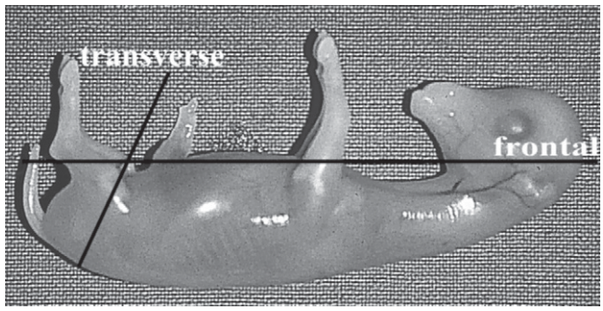
Accuracy of sexual determination can be excellent if an experienced ultrasonographer has optimal visualization of the fetus. Ideal examination conditions should include a high-quality ultrasound machine with at least a 5 MHz transducer on a transrectal probe, low external light, ultrasound monitor positioned at eye level close to the examiner, a cooperative mare that will stand still with minimal rectal straining, and the opportunity to repeat the examination on a later date if necessary. High-resolution ultrasound transducers (7.5 to 10 MHz) with an adequate depth of penetration will enhance the visualization of the fetal parts. An ultrasound machine equipped with a cine feature will allow the operator to review previously scanned images frame by frame. The accuracy of the diagnosis is not only dependent on the examiner confirming the presence of the genital tubercle in the appropriate position for the sex of the fetus, but also on the determination that the genital tubercle is absent from the genital position of the opposite sex. If both of these conditions are not imaged repeatedly during the examination, the accuracy will be less than optimal.

The entire fetus should be observed and the position of the beating heart, anechoic stomach, tail, limbs, and umbilical cord noted. Although a sagittal section is useful to identifying body parts and to determine the orientation of the fetus, it is not particularly useful for identifying the genital tubercle. The presence or absence of the genital tubercle in a frontal plane, and a transverse cross section positioned through the inguinal region of the fetus should be determined (Fig. 11-7). A positive male view and a negative female view must be imaged to confirm that the fetus is a male. Conversely, a positive female view and a negative male view are necessary to confirm that the fetus is a female.

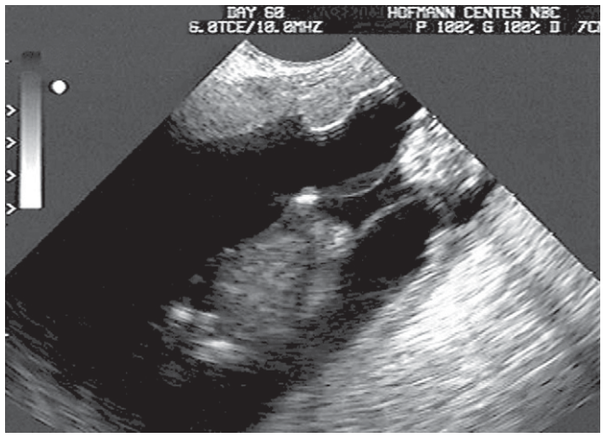
Late Gestation

Assessment of the late gestation conceptus is limited with ultrasonography per rectum because only the cervix and only a small amount of the caudal uterine contents can be visualized. During the first two trimesters of gestation, the fetal fluids tend to be nonechogenic; however, in the last part of pregnancy, it is normal for a slight to moderate amount of echogenic particles to be dispersed throughout the allantoic fluid. After a period of fetal activity, swirls of echogenic material can be seen in the amniotic and allantoic fluids. Excessive amounts of echogenic particles can indicate placentitis, dehydration, or fetal diarrhea.

The uteroplacental unit, which consists of the chorioallantois and the endometrium, tends to increase in thickness as gestation progresses. Normally the uteroplacental unit is less than 12 mm thick even near term. A uteroplacental thickness greater than 12 mm may be associated with placentitis, placental edema, or placental separation. Small areas of separation between the chorioallantois and the uterus can be seen in an uneventful, normal pregnancy. Near term the chorion immediately over the cervix may separate slightly as the cervix relaxes. It is not unusual for the chorioallantois sur-



A



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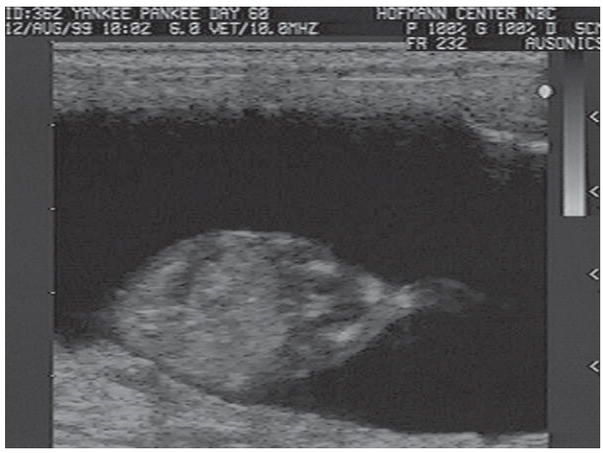
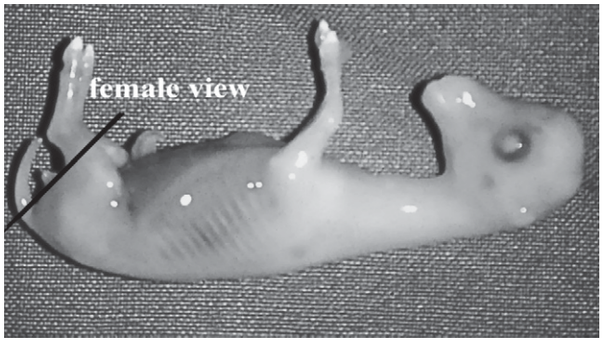
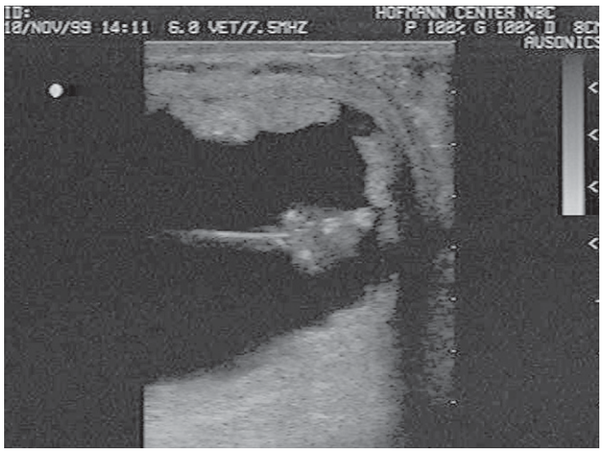


C



D



E

Fig. 11-7 **A,** The ultrasound probe should be positioned over the fetus to direct the ultrasound beam in a frontal and transverse cross section through the genital tubercle. **B,** A frontal section of the ventral aspect of male fetus allows imaging the forelimbs, ventral abdomen, genital tubercle, and the hind limbs. **C,** Transverse cross section of a male fetus perpendicular to the spine and caudal to the umbilical cord will allow imaging of the abdomen and the genital tubercle. **D,** The ultrasound probe should be positioned to make an oblique cross section through the pelvis to allow visualization of the tailhead/sacrum, femurs/proximal tibias, and genital tubercle in a female. **E,** A caudal oblique cross section though the tailhead and proximal tibias will produce an image that appears like a triangle surrounding the centrally located genital tubercle in a female.

rounding the insertion of the umbilical cord to be separated from the endometrium. Extensive areas of fetal membrane thickening and separation of the chorioallantois in the uterine body (Fig. 11-8) may signal impending premature separation of the fetal membranes at the time of parturition.

Fortuitous identification of the round anechoic orbit of the fetus during an examination using ultrasonography per rectum indicates cranial presentation. Ranges of orbit diameter, rib diameter, intercostal space, and trunk, stomach, and brain case size in Thoroughbred and Standardbred mares have been established to permit estimation of fetal age.[6]

After 120 days of gestation, transabdominal ultrasonography can be used to assess the intrauterine environment and fetal status.[7] Number of fetuses present, fetal presentation and position in the uterus, fetal heart rate and activity, and character of the fetal fluids and placenta can be determined. Deviations from the normal profile may indicate the pregnancy is at risk and should prompt preparations to be made for management of a compromised neonate at parturition.

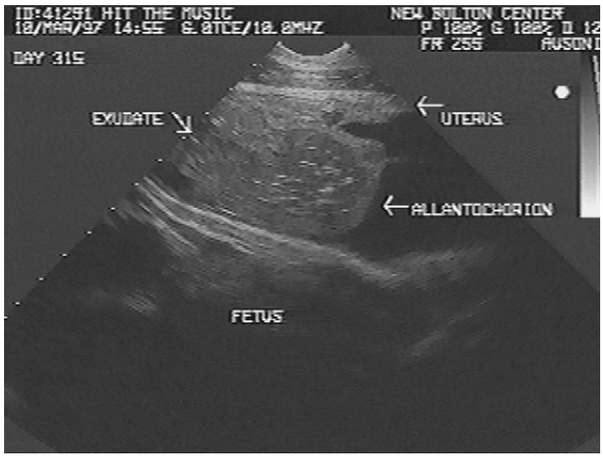


Fig. 11-8 Ascending placentitis. A pool of exudate advanced cranially to separate the allantochorion from the uterine wall of this 315-day conceptus.

ENDOCRINOLOGY

It is critical that the normal endocrinology of pregnancy be understood in order to use endocrine analysis for the diagnosis of pregnancy.[8] Endocrine assays are available, but none are useful at all stages of gestation. One must use these tests at the appropriate stage of gestation and be aware of their specificity so that interpretation of the results will be of clinical value. These tests are most valuable when used in conjunction with other methods of pregnancy detection.

One should check with the laboratory that will perform the assay for their specific guidelines on sample handling. Assays are usually performed using serum or plasma obtained from blood collected in clot (red top) or heparinized (green top) tubes. The sample should be nonhemolyzed and kept cool. Serum or plasma should be harvested from the clotted or heparinized sample shortly after procurement. Samples requiring accurate quantitation should be centrifuged soon after procurement to separate the red blood cells. Aspirated serum or plasma can be frozen and transported on cold packs by express delivery service. Many endocrine laboratories perform equine reproductive hormone analyses. Laboratories experienced with interpreting equine reproductive hormone test results can provide valuable information for the clinical management of broodmares (Box 11-1).

With radioimmunoassay (RIA), serum or plasma samples are mixed with known quantities of radioactive labeled hormone. The amount of radioactivity detected in the sample is measured and that value is compared to a set of known standards of concentrations to determine the unknown concentration in the sample. Radioimmunoassays have specific and accurate results; however, they are time-consuming and require a technician and laboratory capable of handling radioactive materials.

Enzyme-linked immunosorbent assays (ELISA) using highly specific monoclonal antibodies and enzyme-induced color changes allow for fast, easily obtained results. Use of monoclonal antibodies in assays allows for less cross reactivity with related substances than polyclonal antibodies. ELISA may be qualitative or quantitative and simple assay kits are available that can be performed in a veterinary clinic. Expense may be reduced because samples do not need to be transported long distances and laboratories do not need to meet strict regulations regarding use of radioactive materials.

Box **11-1**

Some Endocrine Laboratories That Interpret Equine Hormone Assays

Endocrinology Laboratory
School of Veterinary Medicine
Department of Population Health and Reproduction
University of California, Davis
Tupper Hall, Room 1115
Davis, California 95616 USA
(530) 752-0298 (phone)
(530) 752-6318 (fax)
http://www.vetmed.ucdavis.edu/phr/endolab.htm

BET Reproductive Laboratories, Inc.
6174 Jacks Creek Road
Lexington, Kentucky 40515 USA
(859) 273-3036 (phone)
(859) 273-0178 (fax)
betlabs@aol.com
http://www.betlabs.com

Diagnostic Laboratory
College of Veterinary Medicine
Cornell University
(mail) P.O. Box 5786
Ithaca, NY 14852-5786
(package) Upper Tower Road
Ithaca, NY 14853
(607) 243-3900 (phone)
(607) 243-3943 (fax)
diaglab@cornell.edu
http://diaglab.vet.cornell.edu

PMSG "Equi-Check"
Endocrine Technologies, Inc.
35325 Fircrest Street
Newark, CA 94560-1003
(510) 745-0844; (800) 745-0843 (phone)
(510) 745-0977 (fax)
www.endotech
info@endocrinetech.com

Progestins

Mare blood progesterone concentrations indicate the presence or absence of luteal tissue and are not diagnostic for pregnancy. Progesterone concentrations less than 1 ng/ml at 18 to 21 days after ovulation suggest that a mare is not pregnant. The primary corpus luteum produces progesterone and systemic blood concentrations increase to greater than 4 ng/ml during the first 3 weeks of pregnancy, after which there is a slight decrease. Secondary corpora lutea produce a second rise in plasma progesterone after days 40 to 45, and concentrations plateau in the blood between 65 and 90 days. Thereafter,

progesterone concentrations normally decline so that by 150 to 180 days, plasma progesterone concentrations are negligible (<1–2 ng/ml). At approximately 60 days, the conceptus or fetal placental unit begins to produce progestogens other than progesterone (primarily 5α-pregnanes) that are detectable in the maternal circulation. Results of a specific progesterone assay using gas chromatography/mass spectrometry on pregnant mare blood samples obtained after 60 days of gestation would normally be low.

The antisera used in radioimmunoassay and other immunoassays for progesterone will cross-react with 5α-pregnanes, resulting in an overestimation of progesterone in the blood of mares in late gestation. At present, gas chromatography/mass spectrometry is not available commercially for field use to specifically measure these progestogens. However, less specific assays can measure total progestogens and be of diagnostic value. Any progesterone test should be used in conjunction with other methods of pregnancy diagnosis.

Equine Chorionic Gonadotropin

Chorionic girdle cells of the conceptus invade the mare's endometrium to form endometrial cups. These cups start to produce equine chorionic gonadotropin (eCG) between days 35 and 42. Concentrations of this hormone peak at 55 to 65 days and, as the endometrial cups normally involute, decrease to baseline by 120 to 150 days. If the conceptus dies, the endometrial cups usually maintain their production of eCG until 120 to 150 days of gestation. Accordingly, use of an eCG assay for a pregnancy test has limitations. One must be certain of the breeding date of the mare, and blood should be sampled between 40 and 120 days after ovulation to avoid false negative results. It must be remembered that elevated plasma concentrations of eCG indicate that the mare had endometrial cup formation. False positive results may be obtained if the fetus dies between 40 and 120 days.

Several assay systems have been used to measure concentrations of eCG, including RIA, radioreceptor assay, hemoagglutination inhibition test, polystyrenic agglutination test, ELISA, and direct latex agglutination. The time and technical requirements of the radioimmunoassay and radioreceptor assay preclude their use in clinical veterinary practice. Although only a small amount of eCG is excreted in urine, assays are available for eCG analysis of urine samples. Assay test kits are available to practitioners (see Box 11-1) and may be valuable when used as directed in mares with known breeding dates, particularly in miniature horse mares or fractious mares that will not tolerate palpation and ultrasonography per rectum.

Estrogens

Estrogen is produced by the conceptus as early as day 12, but concentrations of estrogens in maternal blood are not detectable until after 35 days. Estrogens may be bound to sulfates (conjugated) in the serum or plasma or be free (unconjugated). The concentrations of conjugated estrogens are 100 times greater than unconjugated estrogens. Equilin and Equilenin are ring-B saturated estrogens unique to the mare and present in the urine after 4 months of gestation. Estrogens have been measured in feces and urine.

Although serum concentrations of estrone sulfate increase a few weeks after conception, a dramatic increase occurs after 60 days of gestation that can be detected to diagnose pregnancy. After 150 days of gestation, the conceptus produces sizable amounts of estrone sulfate, which are excreted in the mare's urine. Estrone sulfate concentrations not only indicate that the mare is pregnant, but provide information on the well-being of the conceptus. Concentrations of estrogens decrease rapidly if fetal death occurs. Biologic tests have been replaced with chemical assays for estrogens. Radioimmunoassay combined with column chromatography separation was developed to measure low concentrations of estrogens early in pregnancy. Estrogens can be measured in a blood, urine, fecal, or milk sample. Simpler chemical tests are available that can easily measure the high concentrations of conjugated estrogens in urine, including the Cuboni test.

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CHAPTER 12

Retained Fetal Membranes

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The functions of the fetal membranes, also referred to as the placenta, and fluids are to supply nutrition to the fetus, remove waste products from the fetus, exchange oxygen and carbon dioxide between the mare and the fetus, and serve as the major source of protection to the fetus.[1,2] The amniotic fluid, which averages approximately 8 L at term, serves to protect the fetus from external mechanical injury and acts as a bactericidal medium in which the fetus can develop. It also serves as a slippery mucous fluid that prevents the fetus from becoming attached to the developing fetal membranes. The primary source of amniotic fluid is fetal nasopharyngeal secretions and saliva.[1] The allantoic cavity stores fetal urine until such time as it can be removed by the circulation to the mare. Urine enters the cavity through the urachus and reaches approximately 18 L by term. This fluid-filled cavity also acts to protect the fetus against external trauma. Hippomanes are generally located in the allantoic fluid and are formed from sediment and debris plus mucoproteins and calcium phosphate found in urine. Generally at least one hippomane is present but frequently two or three may be found. There is no clinical significance attached to their presence.[1,2]

The fetal membranes must be examined following parturition to evaluate the attachment surfaces that existed during gestation and to determine the appearance of all other aspects, which may be normal or abnormal. The attachment surface of the chorioallantoic membrane is an excellent indicator of the endometrial surface during that gestation and possibly future pregnancies. Examination of the fetal membranes during gestation is also possible using ultrasonography and can be used to aid in the diagnosis of placentitis. Routine pregnancy examinations are performed by rectogenital examination at greater than 50 days of gestation unless there is reason to suspect a problem with the pregnancy. Indications of problems include vulvar discharges, premature udder development, edema of the ventral abdominal wall, illnesses or injuries of the mare that may affect the fetus and the pregnancy, extreme enlargement of the abdomen, and in some instances, prolonged gestation. In addition to evaluation of the fetal membranes during ultrasonographic examination, the fetus and fetal fluids should be examined. The thickness of the near-term amniotic membrane is approximately 2 mm.

Postpartum examination of the placenta is an excellent diagnostic aid, because the expense is minimal and the evaluation can be rapidly performed. The membranes can be inspected during the same visit in which the foal is examined. If the environmental temperature is high, the placenta should be refrigerated until it is examined to preserve many important characteristics. The first characteristic to observe is the location of the chorionic side of the membranes. This side should be inverted, with the allantoic side outermost, if the delivery was normal. The chorioallantoic membrane should be examined for thickness, absence of villi, and any area of abnormal color or appearance. The weight of the placenta, although previously stressed as being important, is probably the least significant placental characteristic and the author has chosen to eliminate this portion of the examination because of a lack of correlation of placental weight to any specific problem.

The next part of the examination is to carefully visualize the entire surface of the chorioallantoic membrane and place it in the T shape that normally exists; the gravid horn makes one side of the T much larger than the other. Several small sacculations may be present near the junction of the pregnant horn and body of the uterus on the allantoic surface. These areas contain sloughed endometrial cup material.[1,3] Another sacculation may be attached to the umbilical cord and can be fluid-filled and up to 20 cm in length.[4] The umbilical cord is composed of two arteries, a vein, and the urachus, plus lymphatics. One artery originates from each of the two horns.[2] Twisting of the umbilical cord is normally very common and makes the diagnosis of umbilical torsion and occlusion somewhat more difficult than it is in other species. The normal approximate length of the twisted umbilical cord is 50 to 60 cm.[1,2] It has been proposed that shorter-than-normal umbilical cords can result in umbilical herniation. It has been reported that umbilical cord torsion can result in vascular occlusion and urachal occlusion, which may result in death of the fetus and abortion or previous urachus and predisposition to rupture of the bladder.

The next portion of the examination is the amnion. This is examined to determine whether meconium is present. Meconium presence generally indicates a problem with fetal oxygenation resulting in fetal distress near term or partutrition.[1] The chorionic side of the allantochorionic membrane can be examined in detail following inversion so that the allantoic portion is once again internal to the chorionic surface. This examination provides an evaluation of the mirror image of the attachment surface that existed during gestation and cannot be replaced by any other diagnostic test. The surface should ideally be roughened with the presence of villi except where the papillae of the oviducts enter the uterus, where placental folds exist because of overlapping in the areas adjacent to the endometrial cups, where the yolk sac attachment existed, and where the internal os of the cervix was located.[4] Any abnormal condition such as

endometrial fibrosis, placentitis, or uterine cysts can reduce the attachment surface available and may be sufficient to result in fetal growth retardation or loss.[5] The tips of the chorioallantoic membrane near the apical end of the uterine horns are frequently edematous, with the gravid horn more often affected than the nongravid one.[1,4]

Placentitis caused by infectious organisms may account for up to approximately 16% of equine abortions. Streptococcal organisms were reported to be the most likely cause of placentitis, representing approximately 23% of those diagnosed. *Escherichia coli, Pseudomonas* spp., *Klebsiella* spp., and *Staphylococcus* spp. have also been reported as important causes of bacterial placentitis.[6] Fungal infections of the fetal membranes account for approximately 41% of abortions reported by several investigators. Viruses account for the remainder of the infectious placentitis causes of abortion.[1,2,6] Organisms enter the uterus through the cervix or by hematogenous spread. Organisms that enter by the cervical route include those that enter the uterus prior to or at the time of breeding and do not manifest themselves as a clinical problem until abortion. Ascending infections may result in lesions located near the internal cervical os.[1,6] Less than normal vulvar conformation can result in bacterial contamination of the vagina and cervical inflammation. This may cause the cervix to relax and bacteria or fungi to enter. It has also been suggested that cervical relaxation may also occur during transition of pastures from poor to lush vegetation. Bacterial infections that are acute in onset result in abortion, whereas chronic infectious often result in fetal growth retardation or emaciation.[1] Lesions associated with infections of the placenta include inflammation and thickening. The nature of the amniotic fluid may prevent spread of bacterial organisms directly to the fetus, but entry is possible by invasion through the umbilical vessels and cord.[6]

There was reportedly an increase in mycotic placentitis associated with the use of intrauterine antibiotics and with increased manipulations, which result in entry of these organisms into the reproductive tract. The chorioallantoic membrane may increase in thickness by five to ten times.[6] The amnion is involved in fungal infections in only approximately 10% of cases, and the fetus may have the characteristic appearance of thickened and roughened skin. Severe placentitis accompanying fungal infections restricts nutrient supply to the fetus and reduces fetal size.

Retention of the fetal membranes is reported to be the most common problem in postpartum mares. Authors consider failure of passage of part or all of the allantochorionic membrane with or without the amniotic membrane within a predescribed period of time as retention of the fetal membranes. The length of time varies among authors from 30 minutes to 6 to 12 hours.[2] Retention of fetal membranes has also been described as retained placenta or retained afterbirth. This condition is reported to occur with a frequency of 2 to 10.5%. It is difficult to determine from the literature an exact incidence because variation exists in the point of time post partum at which a placenta is considered to be retained. Retained placenta is more common in draft breeds than in medium- or lightweight breeds. Following dystocia or prolonged gestation, there is also an increase in the incidence of retained fetal membranes. Dropsy of the fetal membranes and cesarean section have been related to an increased incidence of retained fetal membranes. There is disagreement between authors as to whether there is an increased incidence of retained fetal membranes following abortion, stillbirth, or twinning in lightweight horses. It appears that there may be no increase unless these conditions coexist with dystocia.

A more recent study found the following relationships to retained fetal membranes: mares that were bred naturally versus those that conceived by artificial insemination have an increased incidence of retained placentas when the breeding occurred at foal heat.[7] There was no reported difference in placental passage in mares artificially inseminated at foal heat versus mares artificially inseminated at second, third, or later postpartum estrus. In mares that were barren or maiden or had foaled the previous year, there was no difference in the incidence of retained placenta following parturition. In mares older than 15 years of age there was a higher incidence of retained fetal membranes than there was in mares that were younger.

Time of year appeared to influence the incidence of retained fetal membranes only on certain farms, with a higher incidence after March 31. The sex of the foal did not influence the incidence of retained fetal membranes. There was no reported difference in the retention incidence between the birth of weak or diseased foals and that of healthy foals. During normal parturition, separation occurs between the allantochorionic membrane and the endometrium during stage III of labor. The mechanism responsible for separation is believed to involve cessation of blood flow through the umbilical cord and the corresponding collapse of fetal placental vessels and decrease in size of chorionic villi.[2] This is followed by uterine contractions, which enhance reduction of uterine size and separation of chorionic villi. Uterine horn contractions originating nearest the ovary and progressing toward the body of the uterus aid in separation and expulsion of fetal membranes from the uterine lumen. As the placenta enters the cervix, its presence enhances the release of oxytocin and results in increased uterine and abdominal contractions. Once the placenta passes between the vulvar lips, the weight of the placenta aids in expulsion by applying gentle pressure and stimulating separation.

Causes of retained fetal membranes are uncertain at the present time. A variety of situations or circumstances are related to retention of fetal membranes, but no specific single cause has been described. It is known that complete separation of the placenta cannot occur until the microvilli are released from the maternal crypts. These microcotyledons are firmly attached to the endometrium by the seventh month of gestation. It has been suggested that in mares with retained fetal membranes, retention is not necessarily caused by attachment of the fetal membranes in both uterine horns but rather in the previously gravid horn or the nongravid horn. An additional report suggests that attachment and failure to separate does not necessarily occur in either of the horns

but actually occurs in the body of the uterus. It appears, however, that the majority opinion favors the failure of the previously nonpregnant horn to separate from the fetal membranes. Characteristics of the placenta that may account for the difference in horn separation include variations in allantochorion thickness, length of the villi, and the degree of attachment of the fetal membranes in the gravid horn or in the nongravid horn. Furthermore, because of the success of oxytocin therapy in mares with retained fetal membranes, it has been speculated that a hormonal imbalance accounts for membrane retention. It has been suggested that more microvilli are present in the uterine horns than in the body of the uterus and that these microvilli are much more branched and larger than in the nonpregnant horn. Reportedly, folding of the fetal membranes and the endometrium occurs more commonly in the nongravid horn than in the previously gravid horn or body of the uterus. Uterine involution may occur at a slower rate in the nongravid horn than in the previously gravid horn or in the uterine body. It is possibly one of these phenomena, a combination of two or more, or unknown events that are responsible for retention of fetal membranes in mares. Furthermore, the edema of the placenta may be partially responsible for retention. Edema apparently is more prevalent in the gravid horn than in the nongravid horn, which would add support to the hypothesis that the gravid horn is the primary site of fetal membrane retention.

Endometritis or inflammation of the uterine lumen during pregnancy has been suggested as a possible cause of fetal membrane retention. Inflammatory responses could create adhesions between the chorioallantoic membrane and the endometrium and delay separation of the fetal membranes. These infections could be introduced during breeding, could enter the uterus through the cervix during gestation, or could spread to the uterus via the blood. In addition, it has been postulated that air entering the uterus immediately following expulsion of the fetus could be a source of bacteria and other substances that could be responsible for a subsequent inflammatory response.[2] It is difficult to believe, however, that substances entering the uterus at the time of parturition could create circumstances that lead to endometrial inflammation, placental edema, decreased effectiveness of oxytocin, or any of the other possible causes of retained fetal membranes within a few minutes following parturition. The theory that bacteria enter the uterine lumen at breeding and remain dormant for a prolonged period of time appears to have more credibility than that of bacteria that enter at the time of parturition in regard to an effect on fetal membranes. It has been reported that infections occurring between the fetal membranes and the endometrium are much more prevalent near the external os of the cervix and the surrounding area of uterine body than at any other location within the uterine lumen. Because this area is rarely involved in fetal membrane retention, it appears that infection as a cause of fetal membrane retention is not a high probability.

Mares with retained fetal membranes reportedly do not exhibit the usual signs of colic and abdominal straining immediately post partum that occurs in mares that pass the placenta normally. If a hormonal imbalance were responsible for failure of myometrial contractions that normally occur after foaling, this would account for a decreased occurrence of straining and abdominal pain. Furthermore, excellent results have been reported with oxytocin in the treatment of retained fetal membranes.[8] Oxytocin normally increases in the blood during the second and third stages of a normal parturition. Furthermore, oxytocin has been used to induce parturition, and increased doses reduce the time to the second and third stages of labor.

It has also been suggested that uterine inertia can be caused in mares by a decrease in the concentration of blood calcium; overstretching of the myometrium, which is seen in cases of hydrops allantois, twinning, or oversized fetuses; and in cases of myometrial degeneration resulting from bacterial infection. Uterine inertia has also been incriminated following premature deliveries when the proper sequence of hormonal interaction has not been established. In one mare with retained fetal membranes, high concentrations of progesterone persisted, in contrast to the decrease observed in unaffected mares. Relaxin concentration was reported to remain elevated in a mare with retained fetal membranes. It has been suggested that the presence of retained fetal membranes within the lumen of the uterus serves as a continued source of relaxin. The function of prostaglandin $F_{2\alpha}$ remains unknown in the normal physiologic passage of fetal membranes.

Clinical signs of retained fetal membranes primarily consist of a portion of the membrane protruding from the vulvar lips. The degree of membrane protrusion varies from a few centimeters to a situation in which the placenta protrudes from the vulva to the ground. Retained fetal membranes can be present without any visible signs. In affected mares, the entire fetal membrane or a portion thereof is retained within the uterine, cervical, or vaginal lumen. Diagnosis in these cases is more difficult than in those in which the fetal membranes are apparent. Retained fetal membranes that protrude from the vulva generally create no adverse side effects unless the placenta irritates the mare and causes her to kick at the placenta. Some mares unintentionally kick their foals while trying to get the placenta away from their hocks. For this reason it is suggested that the placenta be tied in a knot ventral to the vulvar lips. Another suggestion has been to place the placenta in a plastic sleeve and tie it so that the placenta is included inside the plastic sleeve. This can be done in such a way to keep the placenta from touching the hocks or distal extremities.

The complications most frequently reported following retained fetal membranes include metritis, laminitis, septicemia, and death of the mare.[9] Acute metritis and laminitis appear to be more of a problem in the draft breeds than in the medium- to lightweight breeds. It has also been suggested that the risk of uterine infection and delay in uterine involution increase with the duration of placental retention. This observation resulted in veterinarians removing the retained fetal membranes manually from the mare's uterus. This is no longer a recommended treatment. The relationship between laminitis and retention of the fetal membranes was thought to be the result

of the delay in uterine involution because the environment is suitable for bacteria to multiply within the uterine lumen and result in absorption of bacteria and toxins.

Many types of gram-negative organisms capable of producing endotoxin and causing histamine release have been found in the uterus with retained fetal membranes.[2] In a report of 356 Standardbred mares with retained fetal membranes in which the fetal membranes were not manually removed, there were no reported cases of acute metritis, septicemia, toxemia, or laminitis during or following passage of the placenta.[7] It should also be noted that postpartum laminitis can occur as a result of systemic acute metritis with or without a retained placenta.[10] Septic or toxic metritis can result in systemic signs of elevated body temperature, changes in disposition, and changes in appetite. However, toxic or septicemic metritis can occur in the absence of obvious retained fetal membranes. There may be retention of many microvilli in large areas where they have broken free from their attachments and remained embedded in the maternal crypts of the endometrium. This is the primary reason that manual removal or attempts to separate retained fetal membranes from the endometrium should be avoided.[2] Although a major portion of a retained fetal membrane could be removed manually, the large number of remaining microvilli can serve as foci of infection and inflammation with fluid accumulation within the uterine lumen. It is imperative that following expulsion of fetal membranes the chorionic surface be examined carefully to determine its gross appearance.

Laminitis may occur when large numbers of microvilli remain attached to the endometrial surface, which is followed by systemic involvement from septic uterine contents or related to histamine release by the uterus. Laminitis has been attributed to degenerative changes that occur within the tissue between the third phalanx and the hoof wall. This is in contrast to the normal sequence of events, which include toxic capillary injury and edema. The substances produced within the uterus may also have a direct effect on the sensitive lamina of the hoof. The specific mechanism involved in the development of laminitis following septic metritis is not known. Two possibilities have been suggested that involve a vasoactive component and a coagulation component. The relationship of hormones or toxins acting on digital vessels and thus altering the circulation of blood to the feet has been included in what has been known as the vasoactive component. Furthermore, it has been suggested that peripheral blood circulation increases immediately postpartum because of a reduction in uterine size and increase in uterine tone, thus moving blood from the uterus to the peripheral circulation. It is suggested that this is the vasoactive component of laminitis. The coagulation component is thought to be related to intravascular coagulation and fibrogen degradation products; thus, treatment with heparin has been recommended to reduce the incidence of laminitis.

Laminitis associated with retention of fetal membranes or, more specifically, with toxic or septic metritis, is similar to that which occurs following gastrointestinal involvement. The initial signs of laminitis occur 2 to 4 days following the onset of septic or toxic metritis. At that time, the animal exhibits an unusual gait and unusual stance by attempting to bear more weight on the rear limbs than on the forelimbs; thus, the rear limbs are placed further forward under the animal than normal. In such a case, the hooves of the horse are warmer to the touch than normal and a characteristic pulse may be present in the digital artery. Many affected animals spend the majority of their time lying down or stationary in a standing position.

The literature suggests that any animal with retained fetal membranes be treated to prevent laminitis. This includes the use of ice or cold water foot baths, suitable footing, and intrauterine and systemic antibiotic therapy to prevent septic metritis. Substances such as antihistamines, phenylbutazone, nonsteroidal anti-inflammatory drugs, and tranquilizers have been included in recommended therapy for retained fetal membranes. Antihistamines apparently neither prevent nor cure laminitis following retained placenta and their use is not recommended. It has also been suggested that the quantity of roughage be reduced in animals demonstrating signs of laminitis and that grain be completely eliminated from the diet.[2] Some authors recommend flushing the uterus when septic or toxic metritis is present or manually removing the placenta immediately to reduce the possibility of laminitis.

It is this author's opinion that neither flushing the uterus nor manual removal of the placenta is indicated in mares with uncomplicated retained fetal membranes or in mares affected by septic or toxic metritis. As stated previously, manual removal of the placenta may result in separation of microvilli from the larger portion of the fetal membranes. In such instances, the microvilli then have to liquefy from the maternal crypts and be expelled or be absorbed by the uterus. Whenever manipulation of the uterus is performed, either for the purpose of manual removal of retained fetal membranes or in uterine lavage, there is an increase in blood supply and potentially an increase the absorption of substances from the uterine lumen into the circulatory system. If these substances are of a septic or toxic nature, manipulation of the placenta or flushing of the uterus generally initiates a deterioration in the mare's general clinical condition. Such treatment may be more detrimental than beneficial. Excellent results have been obtained with conservative therapy involving systemic medications, including antibiotics and supportive care when indicated during the first 2 days post partum. The conservative approach in treating retained fetal membranes permits the mare to establish the normal physiologic barrier that is responsible for prevention of septic or toxic signs following parturition in most mares. This supportive care may include systemic antibiotics, anti-inflammatory drugs such as phenylbutazone or flunixin meglumine, and systemic fluids if required. This approach to treatment of retained fetal membranes generally results in improvement in the mare's condition within 24 to 48 hours. Once improvement has occurred, local treatment of the uterus may be initiated. Frequent measurement of body temperature

and complete blood counts in systemically affected mares demonstrates subclinical relapses following manipulation of the uterus for examination, uterine infusion, or uterine lavage.

Treatment of retained fetal membranes varies widely and each has its advantages and disadvantages. The first reported treatment for retained fetal membranes was manual removal. A variety of methods are described in the literature for manual removal of fetal membranes from the uterus of a mare. These methods include grasping the free portion of the fetal membrane protruding from the vulvar lips and applying traction; inserting the hand between the chorion and the endometrium to force separation of the two; placing the hand between the chorion and the endometrium, applying massage rather than simply using the hand as a wedge to separate the two structures; grasping the chorioallantoic membrane and twisting it, thus forcing separation of the fetal membranes from the endometrium; and, possibly the most complex, placing a wooden ring over the fetal membranes and forcing it between the membranes and the endometrium.[2] It has been recommended that the time between parturition and manual removal be anywhere from immediate to 24 hours. The primary rationale for early removal is to avoid delay, thus reducing the time required for uterine involution and reducing the possibility of uterine infection. In the same report, however, the author states that manual removal of fetal membranes delays uterine involution. Several vaginal examinations at 4- to 12-hour intervals may be necessary to manually remove fetal membranes without causing injury to the mare. It was furthermore suggested that the membranes be manipulated no longer than 10 minutes at each visit.

Although manual removal has been reported to be the treatment of choice by many authors, it is accompanied by several undesirable complications, the first of which is severe hemorrhage. Mares can lose large quantities of blood into the lumen of the uterus following manual separation of the fetal membranes. Assuming that the mare survives, this blood serves as an excellent environment for bacterial growth. It has also been reported that pulmonary emboli follow manual removal of the placenta. Manual removal of fetal membranes has been accompanied by invagination or eversion of a uterine horn, as well as a delay in uterine involution. It has been reported that there is an increased amount of fluid accumulation in the uterine lumen following manual removal of fetal membranes. The primary reason for the increase in fluid is that during separation only the central branches of the chorionic villi within the maternal crypts are removed. The remainder of the microvilli are broken off and retained within the endometrium. Furthermore, rupture of the endometrial and subendometrial capillaries occurs adding fluid within the lumen of the uterus. The presence of microvilli remaining within the endometrial crypts has been related to an increased occurrence of endometritis, laminitis, uterine spasm, and delayed involution. One author acknowledged that manual removal should be discouraged because of the possibility of trauma, hemorrhage, and infection. Manual removal was then recommended after other methods failed. It has been postulated that manual removal of the fetal membranes can result in permanent endometrial damage. The author has observed an increase in endometrial fibrosis occurring in a number of mares from 3 to 14 years of age that had normal fertility until manual removal of the fetal membranes. Following manual removal, decreases in fertility and deterioration in endometrial biopsy classification due to an increase in endometrial fibrosis were observed. The cervices of mares in which the fetal membranes have been manually removed remain open longer than those of mares treated with more conservative approaches. It has been suggested that manual removal of the fetal membranes remains as a treatment from a time when draft horses predominated and retained fetal membranes were more of a problem. The use of antiseptics placed into the lumen of the uterus has been suggested as an alternative method of treatment. Povidone-iodine diluted in large volumes of warm water has been placed into the lumen of the uterus or inside the placenta. Another method describes the placement of 2 to 12 L of dilute povidone iodine solution into the allantochorionic space followed-by ligation of the cervical star. This procedure traps the fluid within the allantoic cavity[9] and stretches the uterus and dilates the cervix as the placenta is passed, thus initiating endogenous oxytocin release and separation of microvilli from their attachments. It is suggested that the placenta should be passed within 30 minutes following this procedure. A disadvantage of the use of antiseptics has been their possible depression of phagocytosis by uterine neutrophils. The clinical importance of this phenomenon is unknown. Flushing and siphoning of fluid from the uterus is reported by some authors to be successful in the management of retained fetal membranes but by others to be of questionable value.[2] Infusion of the uterus with warm water (42° C) has been used with beneficial results. Large volumes of intrauterine saline and uterine flushes containing antibiotics have reportedly prevented or cured laminitis. Much diversity with regard to uterine therapy obviously exists. The most conservative treatment for retained fetal membranes in the author's opinion is the use of oxytocin. A variety of doses and routes of administration are described in the literature. The use of oxytocin in a slow intravenous drip with 30 to 60 units placed in 2 L of saline and administered over an hour or of 80 to 100 units added to 500 mL saline and administered over a 30 minutes were both successful. Oxytocin can be administered as a bolus intravenously or intramuscularly. Doses for bolus injection range from 20 to 120 units. The administration of oxytocin can be repeated at 1.5- to 2-hour intervals until the placenta is delivered. Owing to the extremely short half-life of oxytocin, there is no detrimental effect of repeated administration. The only disadvantages of administration of a bolus are the spasmodic myometrial contractions and abdominal straining that may follow. It has also been reported that signs of colic appear more frequently when oxytocin is administered as a bolus.[8] The severe cramping and abdominal straining may be related to dose. This author has not observed colic requiring treatment following administration of 20 units of

oxytocin intramuscularly at 1- to 2-hour intervals. Furthermore, if signs of colic do occur, they can be eliminated with sedatives or analgesics. As the interval between parturition and treatment increases, the dose of oxytocin may be slightly increased. Dosages administered shortly after parturition should not exceed 20 units. Oxytocin is effective up to at least 18 hours post partum. As the animal approaches 15 to 18 hours post partum, the dose of oxytocin may be increased to 40 units per injection. The route of administration does not appear to affect the clinical outcome.

Antibiotics have also been recommended in the treatment of retained fetal membranes. It is probable that antibiotic treatment does not result in a more rapid expulsion of retained fetal membranes but primarily aids in the prevention of metritis. One report indicated the successful use of oxytetracycline hydrochloride in capsule form. In this author's experience, the use of powered antibiotics or antibiotics in capsule or tablet form is undesirable because these antibiotics must be dissolved before they can have contact with the endometrial surface. Liquid preparations are believed to be superior for intrauterine administration. Other antibiotics that have been used in treatment of retained fetal membranes include amikacin, polymyxin, ticarcillin, sulfanilamide, and penicillin. It has been recommended that intrauterine antibiotics be administered at approximately 8 hours post partum if fetal membranes are retained. The primary value of antibiotic treatment is reportedly to control the contaminating bacteria that enter the reproductive tract at the time of parturition. Furthermore, it has been suggested that the fetal membrane attachment to the endometrium be examined prior to each treatment. Mares treated with intrauterine antibacterial agents for placental retention have a significantly higher pregnancy rate at the end of the breeding season than do mares that have not been treated with intrauterine antibiotics.[7] The incidence of abortion, however, is higher in this group than in mares that have not been treated with intrauterine antibiotics. There was no difference in the foaling percentage of mares treated or not treated with intrauterine antibiotics for retained fetal membranes. Another reason not to incorporate intrauterine antibiotic therapy as part of a "routine" treatment for retained fetal membranes is based upon a report indicating that by 5 hours following intrauterine administration of antibiotics, 45% of the mares expelled the fetal membranes, compared with 81% of mares treated with oxytocin alone.[7]

Protection against tetanus must always be a consideration following parturition but especially in cases of retained fetal membranes. The use of tetanus antitoxin or toxoid is recommended. The placement of sutures or metal clips in the dorsal vulva following parturition if indicated has been recommended to reduce uterine contamination.[2] The beneficial aspects of intrauterine therapy or the need to manually remove the placenta rapidly has not been established. It is established, however, that systemic oxytocin treatment for uncomplicated cases of retained fetal membranes is superior to all other forms of therapy. A heavy hunter mare that was treated only with systemic antibiotics following a cesarean section passed the placenta 5 days following delivery with no adverse side effects.[11] The animal was not given intrauterine medication because of her disposition. Another report involving a mare first examined 5 days following parturition indicated that she had passed the placenta on that day without signs of complications, having received no prior therapy. This author treated a mare following an abortion at 9 months of gestation with intrauterine oxytetracycline once daily for 3 days followed by intrauterine infusion of 2% Lugol's solution every other day until passage of the fetal membranes. This mare received phenylbutazone for the first 4 days of treatment. The fetal membranes were passed 13 days following the abortion. The uterus was cultured 34 days following delivery and contained no bacteria. The mare was bred approximately 60 days following abortion and conceived, maintained the pregnancy, and delivered a healthy foal. A careful examination is indicated in all mares with retention of fetal membranes to determine that septic or toxic metritis is not present.

Follow-up examination of mares with retained fetal membranes is mandatory regardless of treatment. It is imperative to be certain that the uterus is involuting normally regardless of whether the mare is to be bred during that breeding season. Transrectal examination of the reproductive tract must be utilized to determine the degree of uterine involution. Additional diagnostic tests may be indicated, including uterine culture, endometrial biopsy, and ultrasonographic examination. Endoscopic examination and endometrial cytology may also be used. Waiting approximately 25 days following placental passage to breed the mare may be beneficial; however, late in the breeding season, mares have been bred at 10 to 20 days post partum following retention of fetal membranes with acceptable conception rates. Examination of the placenta should occur following passage; however, many times it is difficult to determine whether the placenta is complete because of the deterioration of the tissue during retention.

There appears to be no difference in conception rates following the first breeding or at the end of the breeding season. Pregnancy loss rates were similar in normal foaling mares and in mares with retained placenta.[7] This lack of a difference was obvious regardless of the duration of placental retention. Manual removal of the fetal membranes was not attempted. It has been suggested that fetal membranes that cannot be removed easily should not be removed and a more conservative approach should be taken.[12] This author suggests that if fetal membranes have not been expelled by 3 hours post partum, the mare should be treated. The author's treatment of choice is the administration of 20 units of oxytocin intramuscularly every 1 to 2 hours until the mare passes the placenta or until 18 hours post partum. Oxytocin in syringes is dispensed to owners prior to parturition with specific instructions, including contacting the attending clinician at 3 hours post partum so that medication can begin immediately when it is obvious that the placenta is retained. This avoids the delay in the arrival of the veterinarian to the farm, which may not occur until later that day. Mares that foal in the middle of the night should be examined early the following morning to determine if any complications coexist with the retained fetal

membranes or if passage of the membranes has been complete. This examination includes rectogenital palpation and examination of the placenta, if possible. Decrease in uterine tone has been an excellent indicator of potential complications following parturition. If the uterus has good to excellent tone at the time of the examination, no vaginal investigation is made of the vagina or uterus to locate a fetal membrane. If fetal membranes are present within the uterus but good to excellent tone is present, the uterus is infused with 5 to 8 g oxytetracycline in 500 mL of saline. The author is uncertain whether this treatment is necessary. If the uterine tone is diminished, the volume of uterine infusion fluid is increased. In lightweight breeds no other medication is given. In draft breeds or in any mare following correction of a dystocia, systemic penicillin with or without gentamicin and flunixin meglumine is administered. Because mares that have experienced severe dystocias appear to be at a higher risk for developing septic or toxic metritis in conjunction with retained fetal membranes or because of the dystocia itself, systemic medication is continued for 3 to 7 days, depending on the animal's condition. Uterine infusions are changed from oxytetracycline to 2% Lugol's solution on the second to fourth day unless a cesarean section was performed to enhance the contractility of the uterus, thereby increasing tone and decreasing uterine lumen size. The volume of dilute Lugol's solution administered is determined by the size and tone of the uterus and may vary from 500 ml to 4 L. An important consideration regarding the administration of Lugol's solution is to not overdistend the uterus.

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CHAPTER 13

Management of Twin Pregnancy

AMANDA C. RAGON

Twin pregnancy in mares is a significant cause of noninfectious abortion and perinatal death. Twins are reported to account for 20% to 30% of fetal and neonatal tissues submitted for necropsy[1,2] but compose only 0.5% of births.[3] Records of Thoroughbred studbooks in the United States and other countries have confirmed that the prevalence of twins in this breed ranges from 1% to 3.8% of births.[1,4,5] Twins are reported to occur frequently in draft breeds, and foal survival rates are said to be higher than in lightweight breeds,[6] but twins are rare in ponies.[7] At least two authors have reported triplets.[5,8]

Several investigators using ultrasonography for early pregnancy diagnosis have reported much higher rates of twin conception. Twin pregnancies were found in 6.1% of Standardbreds and 15.4% of Thoroughbreds,[6] and a twin conceptus rate of 15.3% was calculated in maiden Thoroughbreds and Standardbreds.[9] A study of German Thoroughbred mares indicated that the rate of twin pregnancies increased significantly and continuously as the breeding season progressed, from 0.9% in February to 1.76% in June and July.

The outcome of twin pregnancy in mares is frequently unfavorable. Of 62 mares carrying twin fetuses, 64.5% aborted or delivered dead foals, 21% delivered a single live foal, and 14.5% delivered both twins alive.[7] Of 124 fetuses in this study, only 18 survived beyond 2 weeks of birth. Mares that abort twins are likely to suffer dystocia, retain their fetal membranes, and experience a prolonged interval to next conception. In contrast, when twin pregnancy is diagnosed and treated while yet in the embryonic stage, 60% of mares deliver a single live foal.[10]

ORIGIN OF TWINS

Equine twins are reported to be dizygotic and arise from multiple ovulations.[11] A set of identical, or monozygotic, twins, has been reported but identification was based on a shared chorioallantois, not on blood or DNA type.[12] In contrast to twins in cattle, freemartinism does not occur in equine heterosexual (colt/filly) twins, although blood cell chimerism has been reported.[6,13]

Multiple ovulations are defined as occurring within a single follicular phase of the estrous cycle.[4] They may be synchronous, ovulating within 24 hours of each other, or asynchronous, occurring up to 48 hours apart. When follicles ovulate more than 2 days apart, the mare's estrous status should be critically examined to determine if each ovulation occurred within the same follicular phase. There appears to be little difference in the twin conception rate at the time of fixation between synchronous and asynchronous ovulations,[4] although in one trial, 96.5% (55 of 57) of mares with twin embryos examined between days 13 and 16 were determined to have had synchronous double ovulations.[9]

Not surprisingly, the incidence of multiple ovulations is highest in those breeds in which twinning is the most prevalent. Besides breed, multiple ovulations are influenced by reproductive status. One investigator found a 50% increase in double ovulations in barren mares over foaling mares.[9] Multiple ovulations are reported by some to occur more frequently during the late spring and summer,[4] although solid support for this conclusion is lacking. There is extensive evidence to suggest that double ovulations occur repeatedly in certain individuals,[4] but the heritability of multiple ovulations is not known. Increasing age has also been associated with a higher risk of twinning.[5]

EMBRYO FIXATION AND REDUCTION

Fixation of the embryo is postulated to happen when the embryos become so large and uterine tone so great that transuterine migration ceases, despite continued uterine contractions.[15] Fixation occurs approximately the same time (day 16 after ovulation) for twin embryos as for singletons; however, ultrasonographic studies found that twin vesicles tended to fix together in one uterine horn (unicornual fixation) more frequently than they were found separately (bicornual fixation). When the size of the vesicles was considered, 85% of embryos that differed in diameter by 4 mm or more fixed unicornually. Similarly sized embryos also showed a preference for unicornual fixation, but by a lesser margin.[4]

Size difference between twin vesicles are likely caused by asynchronous ovulations; thus, a difference in the age of the vesicles. Growth rates of single and multiple embryos are similar, as are diameters of single embryos and those resulting from multiple, synchronous ovulations. Although asynchronous ovulations are not more likely to produce twin embryos,[16] the incidence of unicornual fixation is increased over vesicles resulting from synchronous ovulations.[17]

Embryo reduction refers to the biologic mechanism by which one member of a twin set is eliminated.[4] Evidence

indicates that prefixation embryo reduction is negligible; most reduction occurs after day 16.[18] The pattern of embryo fixation greatly influences whether one embryo of a twin set will be reduced before reaching the fetal stage. Approximately 85% of unicornual twin embryos undergo postfixation reduction, more than half before day 20. The day of reduction appears to be later for unicornual vesicles of similar size.

The "deprivation hypothesis" explains the higher incidence of embryo reduction in unicornually fixed twin sets.[19] According to this theory, embryo reduction, as well as the time of reduction, depends on the amount of contact between the endometrium and the vascularized wall of each embryo. Unicornual twins positioned so that one embryo partially or completely prevents contact between the developing vascular wall and embryonic disk of the second embryo underwent rapid reduction that was complete by day 30. Embryo reduction occurs very late in the embryonic stage, or not at all, in bicornual twin sets and unicornually fixed twins that do not compete for endometrial contact. The chance of embryo reduction decreases and the time to complete reduction increases, the longer a vesicle survives after fixation.

BREEDING MANAGEMENT STRATEGIES: DETECTION AND RESOLUTION OF TWIN PREGNANCY

Historically, twin pregnancy was managed by preventing twin conception. Mares with more than one ovulatory-sized follicle (>30 mm) were not bred in the hope that a single dominant follicle would be present during the next estrous cycle. This is not an efficient method of preventing twin pregnancy, especially within the constraints imposed by the operational breeding season, but it was not critically evaluated[4] and became a common practice.

A 1982 survey of veterinarians documented the widespread practice of withholding mating to prevent twins.[20] Almost all (95%) of the veterinarians surveyed attempted to modify the breeding program in some way, especially early in the operational breeding season. However, the majority of veterinarians responding to a 1988 questionnaire had abandoned this practice and had shifted to early detection of twin pregnancy as a more efficient management technique.[15]

A breeding program that minimizes twin pregnancy but maximizes reproductive efficiency begins with breeding of all mares regardless of number of ovulatory follicles. Breed and other risk factors for twinning should be considered in designing such a program, as well as the intensity and goals of the breeding operation. In addition, a strategic management program should schedule timely examination of mares to detect twin pregnancy, prevent development of twin embryos to twin fetuses, and allow mares that have suffered abortion (spontaneous or induced) to be rebred within the same breeding season. Although ultrasonographic examinations to diagnose twin pregnancy may be less cost-effective for mares at low risk for multiple ovulations, every client should be informed of the possible consequences of undetected twin pregnancy.

Prebreeding Considerations

Ideally, twin management begins before conception. Instituting a reliable record-keeping system enables owners and veterinarians to review a mare's reproductive performance during past breeding seasons, as well as previous estrous cycles during the current season. In addition to recording information on the size and tone of the uterus and degree of cervical relaxation, parameters such as number and size of ovulatory follicles will document trends in a mare's reproductive cycle and identify mares that experience multiple ovulations. Because not all multiple ovulations are identified by transrectal palpation, ultrasonography may be used if there is any uncertainty that ovulation has occurred.

A prebreeding ultrasonographic examination of the uterus identifies the presence, location, number, and size of any uterine or endometrial cysts. Although cysts rarely decrease fertility (unless multiple or very large), they are commonly misidentified as early "twin" embryos.

Diagnosis of Twin Pregnancy

Ultrasonographic examination of the uterus is essential for accurate diagnosis of twin pregnancy during the embryonic stage. It is critical to perform a thorough, complete scan of both uterine horns and body; a rapid examination is more likely to miss the second embryo from a multiple ovulation. To this end, the patient should be properly restrained (preferably in stocks) and sedated if necessary. Ultrasonography can also be used to identify two corpora lutea in the ovaries.

The first pregnancy evaluation should be scheduled for 16 days after ovulation. If pregnancy is not established, this time frequently coincides with beginning of the next follicular phase and behavioral signs of estrus. Earlier examination (14 days after ovulation) has been suggested by some authors to facilitate treatment of unicornual twin pregnancies.[4,21] Day 14 (prefixation) embryos may be separated from each other at this time and one embryo manipulated to the tip of the uterine horn for manual reduction.

The mare should be examined again at 25 days. At this time, the embryo proper and its beating heart are visible with ultrasonography. This examination is important because it provides an opportunity to diagnose twin embryos that may have been missed during the initial examination, and it allows assessment of embryo viability and growth. If both embryos of a twin set have a visible heartbeat at this stage of gestation, the pregnancy may still be reduced or aborted prior to formation of endometrial cups (days 33–35).[22]

The identification of twin embryos using ultrasonography is well described in several textbooks. Most postfixation embryos are found at the corpus-cornual junction but can occasionally be located further up the horns or in the uterine body. Bicornual twins are generally easier to identify than unicornual embryos but may be confused with uterine or endometrial cysts.

Embryonic vesicles may be similar in size and shape but can be distinguished from cysts because they grow 3 to 4 mm per day.[23] Unicornual twin embryos may be more

difficult to identify than bicornual twins, but may appear larger in diameter than the normal single vesicle and will frequently have a visible membrane.

Management of Twin Embryos

The treatment method chosen to reduce a twin pregnancy depends on fixation pattern and stage of gestation.

Day 16

At the initial pregnancy examination, bicornual twins are easily treated by manual crushing of one vesicle transrectally. Both yolk sacs are located in the uterus and measured by ultrasonography, and the smaller of the two is then crushed between the thumb and fingers. The uterus is then scanned again to document that the yolk sac has been ruptured. This technique is often easier to perform on the uterine horn contralateral to the veterinarian's palpation arm. Success rates have been reported as high as 96% with this procedure. Treatment of mares with antiprostaglandins and progestogens has not reliably improved the outcome of the procedure, but it is the author's practice to routinely administer both following a twin reduction.[24]

Manual crushing of one of a unicornually fixed twin set is more difficult and likely to result in disruption of both embryos. If fixation has not yet occurred, the embryos may be gently separated and one manipulated to the tip of the uterine horn and crushed. After fixation, separation is difficult; however, the majority of these twins will be naturally reduced.[4]

Day 25

The sizes of the concepti and presence of the embryo proper and its heartbeat at this stage of gestation provide information about the viability of each twin. The majority of unicornual twins will have been reduced to a single embryo, or will be undergoing visible signs of reduction. If both vesicles of a unicornual twin pregnancy contain an embryo with a heartbeat, treatment options include (1) induced abortion with prostaglandin (or other methods described elsewhere), (2) transvaginal ultrasound-guided reduction of one vesicle, or (3) nonintervention, in the hope that elimination of one twin will occur before formation of endometrial cups (approximately day 35). When nonintervention is selected, careful monitoring with ultrasonography is recommended every 48 hours until day 33. If there are no visible signs of embryo reduction (decrease in size, absence of heartbeat) by that time, abortion of the pregnancy can be accomplished before endometrial cups are functional, so that the mare can be mated on the next cycle. Return to estrus may be delayed until after day 120 if abortion is induced after the endometrial cups are functional.[22]

Transvaginal ultrasound-guided twin reduction appears to be most successful when attempted before 36 days' gestation. This procedure utilizes a 5-MHz endovaginal curvilinear transducer introduced aseptically into the cranial aspect of the mare's vagina. Once the pregnancy is identified and is isolated transrectally a sterile, 18-gauge 60-cm spinal needle is passed through a needle channel in the transducer casing to penetrate the vaginal, peritoneal, and uterine walls and the allantoic sac. Care must be taken at this point to disrupt only one embryonic sac. Aspiration of allantoic fluid may increase the likelihood of damaging the remaining embryo. Success rates of 50% have been reported by some authors for the reduction of unicornual twins prior to day 35.[31]

Bicornual twins may be reduced manually at day 25, but this procedure is less successful than when performed at day 16.[4] The decline in success rates at this stage has been attributed in part to fluid released from the crushed embryonic vesicle. The fluid surrounds the remaining vesicle and disrupts contact of the vesicle with the endometrium.[32] The use of transvaginal ultrasound-guided allantocentesis to reduce bicornual twins should be more successful, in theory, because the distance between the two embryos makes needle placement less critical. Complete aspiration of embryonic fluid can be completed with little concern of damaging the other embryo. Because fewer sets of bicornual twins are presented for this procedure, the data regarding the procedure success rates and best time to perform are not as plentiful as with unicornual twins.[31]

Management of Twin Fetuses

Twin pregnancy reduction is difficult after the fetal stage is reached. Twins are more likely to abort than to reduce to a single fetus at this gestation.[4] In one study only 6% of mares carrying twins after 40 to 42 days experienced the loss of a single fetus and carried the other to term.[10] Manual reduction of bicornual twin fetuses is technically difficult and unlikely to have a positive outcome.

Several methods of reduction have been described for fetal stage twins. For twin pregnancies greater than 60 days' gestation, transabdominal ultrasound-guided fetal cardiac puncture and injection of potassium chloride has been successful in some mares.[27,28] Patient selection for this procedure is a concern; gestational age apparently influences the outcome, as do many other unidentified factors. The best candidates seem to be mares between 115 and 130 days of gestation, with a recommended upper limit of 140 days. With this stipulation, the success rate is estimated to be around 50%. Administration of flunixin meglumine and altrenogest after this procedure may be of benefit.[27–29] No adverse effects on the mare's health or subsequent fertility have been reported. Macpherson and Reimer modified this procedure by using procaine penicillin for injection into either the thorax or abdomen. They reported the birth of 8 of 13 live foals following this technique.[30]

Another alternative for managing fetal stage twins may be the use of exogenous progesterone. In three mares, supplemental progesterone reportedly prevented abortion during middle to late gestation even after signs of impending abortion were evident.[31] Although the numbers are small, this may be a useful treatment to attempt when twins are first diagnosed during the latter half of gestation and access to the more sophisticated techniques is limited.

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CHAPTER 14
Parturition and Dystocia

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The average gestation length in mares is approximately 340 days. There is considerable variation; gestation length may range from 315 to 400 days and is influenced by season, with pregnancies that terminate in winter longer than those that terminate in summer. When large variation occurs from the average there always should be some concern and the mare should be examined if indicated. The definition of eutocia is a "normal delivery" or as a safe easy delivery without significant injury to either the fetus or the mare.

MARE PREPARTUM CHANGES

Progesterone concentrations in the peripheral plasma are less than 1 ng/ml during the last half of gestation; thus, measurement of this hormone is useless for determining deficiencies or predicting the time of parturition.[1] Other progestogens, 20α-hydroxy-5β-pregnan-3-one and 5β-pregnane-3α,20α-diol, decrease during the last 24 hours prior to parturition.[2,3] Prepartum changes in the mare include enlargement of the udder 4 to 6 weeks prior to delivery. This time varies with the individual animal and is somewhat dependent on the number of previous pregnancies, if any. The udder becomes more rounded as it enlarges. Enlargement and edema of the udder and the abdominal wall immediately anterior to the udder are apparent 2 to 3 weeks prior to foaling. Waxing, or the appearance of a sebaceous-like secretion on the teat ends surrounding the sphincter, should occur as the mare approaches parturition. However, waxing has been observed to occur as early as 4 weeks prior to delivery and may not occur in some mares. Waxing generally occurs with regularity in the same animal at approximately the same time prior to parturition. The teats usually fill and distend 2 to 14 days prior to parturition, but this may not be observed. Some mares leak colostrum prior to parturition. This is undesirable because once the colostrum is lost from the udder it is not replaced. Colostrum should be collected and frozen or substitute colostrum should be made available for the foal following delivery.

Relaxation of the tailhead and pelvic ligaments occurs gradually over several weeks before delivery. During this same period the vulva relaxes and the opening elongates. At 1 to 3 weeks prior to foaling, relaxation becomes apparent in the flank area with the mare appearing somewhat "gaunt" in this area. Approximately 7 to 10 days later, the gluteal muscles and the muscles adjacent to the tailhead relax and appear less evident, with the bones in that area more pronounced. The ultrasonographic character of the fluids within the uterus also changes during the last 10 days of gestation, and echogenic particles appear. The vagina and vulva relax and there is an increase in mucus. The vaginal mucosa may appear reddened; this generally occurs within 24 hours of parturition. The cervix becomes completely relaxed and the uterine contractions increase in frequency and strength. Because some mares foal with only a few of these normal prepartum signs, prediction of the exact time of foaling is difficult without further diagnostic tests. Body temperature has been suggested as a possible method to determine the time of parturition before the onset of stage I of labor.[4,5] Although it has been demonstrated that changes in body temperature measured at 6-hour intervals with an electronic rectal thermometer were not adequate to predict parturition, the use of telemetry with a computerized system to record core body temperatures at 15-minute intervals could be useful in parturition prediction.[6,7]

Several commercial aids are available for use in determining approximately when parturition will occur. These include the Sofchek Test strip, the Titrets Calcium Hardness Test Kit, and the Predict-a-Foal Predictor Kit. The Sofchek Test strips indicate there is a 79% probability of parturition within 24 hours when the hardness test strip exceeds 250 ppm. The Titret Calcium Hardness Test strip indicates a 53% probability of parturition within 24 hours when the hardness exceeds 250 ppm. The Predict-a-Foal Predictor Kit indicates a 59% probability of parturition within 24 hours if the color change is a 4 or greater. These tests are more predictive of indicating when the mare will not foal rather than when parturition will occur.

A number of methods commonly are used to monitor the actual parturition. The most frequently used and oldest method is to stay in the barn with the mare and personally observe. This method obviously has several disadvantages including inclement weather and loss of sleep. This system is not 100% effective, and many people have fallen asleep while supposedly observing the foaling or have gone to the house for a few minutes and have missed the birth. Others systems designed to improve the quality of life of the foaling attendant include video cameras, sound monitors, the Foal-alert system, the Birth Alert system, and other systems designed for the mare at parturition. Video cameras have progressed considerably from the days of the poor image quality requiring a great deal of light and hard wiring. The systems of today require minimal lighting and no wires. The cost of these systems also has been greatly reduced, making them affordable to most horse farms. They permit the person monitoring the mare to remain in a more comfortable environment than would otherwise be possible. The

author has used several video monitoring systems over the past 22 years and has found them to be worth the cost. Sound monitors including baby monitors and intercoms are of limited value because many horse sounds may "wake" a horse owner and not be related to foaling. However, they do work in combination with other systems such as the monitors, which emit an audible signal at the time of parturition. The Foal-alert system is a monitoring device that includes the placement of a transmitter on the vulvar lips such that at the time of parturition the lips spread with passage of the fetus and the transmitter is activated. The receiver is activated and it produces an audible sound, which can be heard within the barn or transmitted by phone or pager to a distant point. An intercom system can also be used to transfer the alarm sound to a house or sleeping area of the foaling attendant. The Foal-alert system works excellently if any portion of the fetus or fetal membranes filled with fluids reaches the vulvar lips and causes activation of the transmitter. The Birth Alert system is another foaling monitoring system that utilizes a transmitter in the anterior vagina and is activated when the transmitter is expelled from the vagina and the temperature sensor signals the decrease in temperature to the receiver. The receiver transmits an audible signal.

Restlessness increases as the mare approaches parturition. Prior to this increase, mares may demonstrate decreased activity that could last for several weeks. Personality changes are obvious as the time of parturition approaches. This is especially true during the 24 hours before birth. Distractions of any kind should be kept to a minimum during this period. Changes in prepartum myometrial activity have been reported.[8,9] Internally, during this period, the mare's uterus begins more active contractions beginning at the end of the uterus near the ovary and progressing posteriorly toward the cervix.

The fetus is in a variety of presentations until approximately the sixth month of gestation when it aligns itself in a cranial presentation (Fig. 14-1). Presentation may change after this stage, however, and therefore cannot be used reliably to determine the presentation of the fetus until parturition actually begins. The fetus assumes a variety of positions, including dorsosacral, for longer durations as term approaches. As parturition approaches, the head and forelimbs can be palpated in the pelvic canal of the mare and may at times be alongside the cervix with pressure on the vaginal wall.

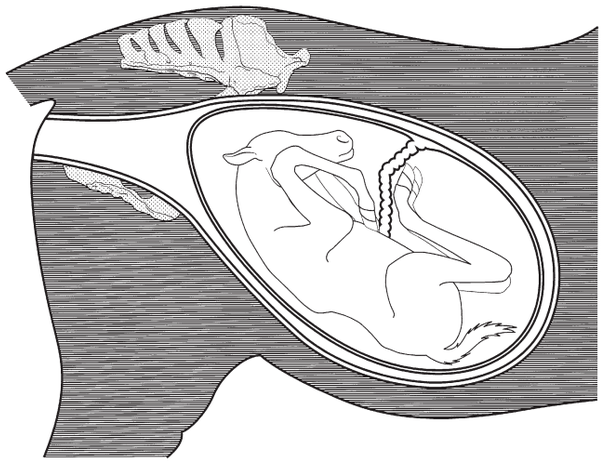

Fig. 14-1 Fetal presentation and posture 2 to 3 months before birth. (From Threlfall WR: *Threlfall's equine obstetrics.* Powell, OH: Global Reproductive Services, 1996.)

There is no need to wrap the mare's tail, wash the perineal area, or wash the udder before foaling. These procedures, although recommended by some, mostly serve to disrupt the mare and have no medical basis. However, cleaning the stall and maintaining reasonable cleanliness are especially important before and immediately post partum. This could include disinfecting stalls between mares but is not practical with most farm facilities. For disinfectants to be effective, all organic material must be removed. This is usually only feasible with a high-pressure sprayer and requires that relatively nonporous material be used in the construction of stalls, including the floor. Stalls should be at least 12 ft by 12 ft or larger and be bedded preferably with large quantities of straw. Other types of bedding material may promote bacterial growth or be too dusty for use with neonates or postpartum mares. If Caslick's procedure has been performed to improve vulvar conformation, the vulva should be opened approximately 14 days prior to the due date or as determined by visual assessment of the mare's preparation for foaling.

Stage I

Sweating initiated in the shoulder area combined with frequent observations of the flank area by the mare are indicators that stage I of labor is occurring. Kicking, biting of the flank, or other signs of mild colic frequently occur during this period. During this stage, final positioning and posturing of the fetus occur. The front feet and the nose make an excellent wedge to aid in cervical dilatation. This is also assisted by pressure of the fetal fluid on the cervical opening. The increase in pressure is first due to increased uterine activity. As the pressure increases and the cervix starts to dilate, oxytocin is released. Oxytocin increases the uterine contractions and indirectly stimulates the abdominal muscles to contract and further increase the uterine pressure. The beginning of stage I of parturition is difficult to determine because its onset is not marked by any single event or change in the mare and is rather vague and variable. As the normal mare approaches foaling, she may have a decrease in activity the day before the night of foaling. It has been reported that approximately 85% of mares foal between 7:00 PM and 9:00 AM, with most mares foaling between 11:00 PM and 4:00 AM.[4] Within 2 hours prior to foaling, the mare may become restless and show signs of colic. She may paw the ground and stop eating for short periods of time as an indication of pain. Increased switching or movement of the tail is also usually observed as the mare approaches parturition. Milk may be seen squirting from the teats. This activity is associated with oxytocin release and apparent uterine pain. Signs of pain are not constant but intermittent and correspond with uterine contractions. These periods may last from 1 or 2 minutes to 20

minutes in length. These events may not be observed in older mares, even though the entire process may take several hours. This explains how mares are able to foal during the 30 minutes an observer may be absent. The mare should be observed but left undisturbed unless problems arise. The average range for this period is 1 to 4 hours. Any disturbances may delay the foaling and result in complications. The chorioallantoic membrane ruptures and the allantoic fluid escapes, marking the end of stage I.

The fetal heart rate prior to the onset of parturition is approximately 76 beats per minute, with elevations occurring with increased activity. Heart rate may increase by as much as 40 beats per minute and the accelerations may average one per minute. The activity of the fetus is most pronounced during the period of 5 to 72 hours prepartum. The fetal heart rate during the 24 hours prior to delivery is approximately 62 beats per minute and during stage I and stage II is between 54 and 60 beats per minute. The heart rate of the neonate averages over 100 beats per minute.

Stage II

Signs of the second stage of labor are more apparent. The mare lies down during most of this period; although she may sit up and lie down several times and is generally sweating. When in the recumbent position, all legs may be extended and the head stretched outward away from the body (Fig. 14-2). She may bite her flank area or chew on hay or straw. During stage II of labor, the mare frequently urinates and defecates because of the pressure applied by the contracting uterus and the abdominal wall and the presence of the fetus and fetal fluids. Some mares may roll in an apparent attempt to lessen the pain or position the fetus. The average time for stage II is 20 minutes, calculated from the time of chorioallantoic membrane rupture until delivery of the fetus. It is abnormal for mares to continue this stage of labor longer than 70 minutes, and prolonged second stage indicates problems are present and fetal death is likely. It is generally recommended that mares be given 20 minutes to complete the second stage before the foaling attendant becomes alarmed. Normal progression in the delivery process should occur during this period.

During stage II, the amniotic membrane protrudes from the vulva (Fig. 14-3). The amniotic membrane should be visible at the vulvar lips within 5 to 10 minutes following rupture of the chorioallantoic membrane if the mare has not been disturbed during the delivery process. Once the amniotic membrane is visible, the fetus has entered the birth canal sufficiently to stimulate strong abdominal contractions. As the foal is pushed caudally by abdominal and uterine contractions, the feet are observed through the amniotic membrane. Rupture of the membrane does not usually occur until the fetus is at least midway or completely through the birth canal. Although most mares foal in lateral recumbency, it is possible for parturition to occur while the mare stands. The major concern in this situation is a ruptured umbilical cord

Fig. 14-2 Mare in stage II of parturition with severe abdominal contraction resulting in all limbs extended. (From Threlfall WR: *Threlfall's equine obstetrics.* Powell, OH: Global Reproductive Services, 1996.)

Fig. 14-3 Mare in stage II of parturition with the forefeet protruding covered by amniotic membrane. (From Threlfall WR: *Threlfall's equine obstetrics.* Powell, OH: Global Reproductive Services, 1996.)

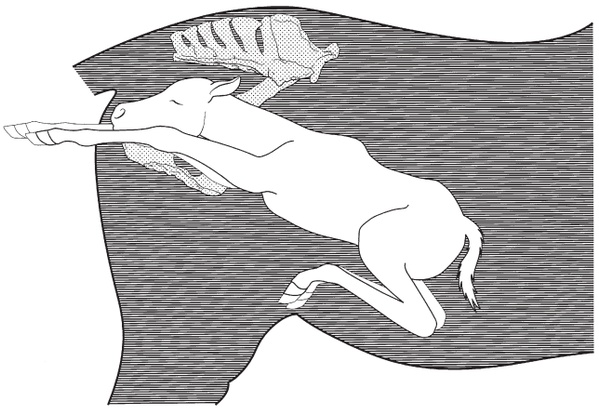

Fig. 14-4 Normal presentation, position, and posture. Anterior longitudinal with dorsosacral position and extension of head, neck, and forelimbs. (From Threlfall WR: *Threlfall's equine obstetrics.* Powell, OH: Global Reproductive Services, 1996.)

close to the abdominal wall and blood loss. If the foal is delivered and the amniotic membrane remains intact as the foal struggles following delivery, the membrane should be incised and removed from the area of the fetal nose and mouth. The normal delivery posture of the fetus is one forelimb extending approximately 4 inches in front of the other and the soles of both feet directed ventrally (Fig. 14-4). This offsetting of the feet results in a slight angling of the shoulders and thereby reduces the width of this area as it passes through the mare's pelvis. While the foal passes through the vulvar lips surrounded by the amnion, the observer can determine the offset position of the hooves and note that the soles are ventral. If the soles are not directed ventrally, immediate attention is indicated. The head should rest on the forelimbs in the area of the carpal joints. The head and neck are extended. If the nose of the fetus has not appeared by the time the carpal joints are observable immediate intervention is indicated. The vertebrae of the fetus are located near to the vertebrae of the mare; the position is close to a dorsosacral but slightly off toward the ileum. This position permits the fetus to take advantage of the greater pelvic diameter. As the fetus is expelled further, the rear limbs are extended so that the hooves are the last part of the fetus delivered. Any variation from this normal position, such as failure to observe one of the feet or the head and neck, indicates a postural abnormality and a difficult delivery. Because the mare's delivery is so forceful, she may severely injure herself by pushing the fetus out and tearing her tissue as the fetus is expelled. Also, anything that slows the delivery process is detrimental because the placenta separates rapidly and eliminates the fetal supply of oxygen. This probably accounts for the poor survival rate of foals when the mare was in need of assistance and none was immediately available.

Stage III

The mare should be permitted to lie quietly in the stall for up to an hour following parturition; however, if she jumps up rapidly following delivery, it is not necessarily a reason to panic. Although it has been suspected, it has not been demonstrated that abdominal wall damage occurs to the fetus when this occurs. The determination of hemorrhage from the umbilicus, although not common, should be made. It was once believed that large volumes of fetal blood could be lost from the placenta and that care should be taken to assure the cord remained attached to the placenta. A more recent scientific study revealed that blood flow through the umbilical vessels ceases by the time the umbilical cord is visible at the vulvar area of the mare.[10] Following delivery of the fetus and after the mare has accepted the foal and is resting, cleaning of the stall and observation of the mare and foal are the two most important managerial tasks. Stage III of parturition begins following expulsion of the foal and ends when expulsion of the placenta is complete. The fetal membranes should be expelled within 3 hours following delivery.

INDUCED PARTURITION

Parturition induction permits observation and professional assistance, if necessary, at the time of foaling. There are primarily two reasons for parturition induction. The first is for medical indications. It is of most value when used for mares that have a history of difficult deliveries or have had injuries or illnesses that could possibly endanger the life of the mare or the foal if not assisted immediately at the time of parturition. Among the clinical indications for induced parturition are ruptures of the prepubic tendon, rupture or weakening of the abdominal wall, following surgical correction of third-degree perineal lacerations or rectovaginal fistulas, previous fracture of the pelvis with narrowing of the birth canal, history of premature placental separation, prolonged gestation, near-term colic, and uterine inertia. Parturition induction also permits opening of Caslick's vulvoplasty immediately prior to delivery and replacement immediately post partum, thereby reducing contamination of the reproductive tract. Furthermore, if an episiotomy is indicated to eliminate vulvar lacerations, it can be performed when indicated. Induced parturition in mares that have previously produced foals with neonatal isoerythrolysis can be easily managed by removal of the foal immediately following delivery.

The second reason for the induction of parturition is for convenience. Induction of parturition is becoming an acceptable method of permitting the owner, manager, or veterinarian to be present at the time of delivery in normal mares. The value of induced parturition in labor savings is brought about by the elimination of "foal watchers," people hired to observe mares near term, or by the elimination of frequent observations throughout the night with the possibility of missing the foaling onset. The labor savings plus the guarantee that capable assistants will be present at the time of parturition easily offset the expenses incurred in parturition induction. The convenience factor also applies to certain research projects involving parturition and neonatal physiology and for the educational value of being able to provide a parturition demonstration. Induction has also been utilized to induce mares to deliver where the primary intention was

to provide a nurse mare for a valuable foal. There is no association between induction and impaired future reproductive efficiency. Owners must be aware of the problems with dysmature or premature foals if delivery occurs prior to the time the foal is mature.

Some of the more general criteria to consider before induction of parturition include knowledge of the gestation length, which should exceed 320 days. The mare's previous gestation lengths can be utilized to some extent to determine term but the effect of time of year should be considered. Mares foaling in the months of January, February, and March have longer gestations than mares foaling in April, May, and June. Prepartum changes such as perineal and cervical relaxation have been used as indicators for predicting parturition but may not be accurate enough to predict the time to induce parturition. Although relaxation of the pelvic ligaments can also be used as an indicator of approaching parturition, it is less noticeable than udder changes and more experience is necessary to detect subtle changes. Mares should be introduced into the foaling environment at least 3 to 4 weeks prior to induction if possible. This permits the mare to be exposed to the organisms in the new environment as well as become accustomed to her new surroundings. The mare should be placed in a clean stall bedded with straw in a quiet area of the barn. Rectal examination of the mare at this time usually permits evaluation of fetal presentation, position, and posture as well as viability of the fetus. Because this is an elective delivery and the time of delivery is predetermined, a tail wrap is applied to the mare and the perineal area is washed prior to administration of the induction agent.

Determination of fetal presentation, position, and posture plus a physical examination of the mare must be performed prior to induction. The fetus should be in cranial longitudinal presentation with slight dorsoileal position and the head and neck extended. Although the final delivery position and posture are determined as the mare enters stage I of parturition, the presentation will have already been predetermined. A caudal presentation would indicate the importance of assistance much earlier in the delivery process than a cranial presentation. If the head and feet are not in the proper posture, it is very probable that correction will occur before delivery without assistance. The author does not advise intervention once delivery has been induced unless it is apparent that the progress is slower than normal or is abnormal. Assistance that is too aggressive or premature may result in cervical lacerations or delay parturition.

Contraindications to parturition induction include conditions that indicate that expulsion of the fetus is not possible through a pelvic canal greatly reduced in size. Any abnormal condition affecting the mare prior to induction should be brought to the attention of the owner and a decision made as to whether to proceed. Conditions such as increased body temperature, abnormal vulvar discharges, and abnormal presentation of the fetus should influence the decision to induce because they may affect the outcome. Fetal extraction following cervical dilation without parturition induction may be indicated in certain instances to provide a better probability of fetal survival.

The most common agent used to induce parturition is oxytocin by intravenous or intramuscular administration.[11] If the cervix is closed, administration of estradiol cypionate (4 to 6 mg) or diethylstilbestrol may be considered 12 to 24 hours in advance of oxytocin administration to aid in relaxation. Oxytocin also has the capability of inducing cervical relaxation in a mare with a closed or nearly closed cervix. The best results are obtained when it is administered as a 10-unit dose intravenously every 15 to 30 minutes by slow intravenous drip. Four to six repetitions of bolus administration may be required. If dilatation occurs without delivery, the dosage of oxytocin can be increased to 20 units. While determining the degree of cervical dilation, one can also determine the position and posture of the fetus by transrectal palpation because disruption of the mare has already occurred.

The dosage of oxytocin administered varies with the degree of cervical relaxation. If the internal diameter of the cervix is relaxed to at least 2 cm, it has been recommended by several authors to administer 40 to 60 units oxytocin as an intravenous bolus. Delivery of the fetus should occur within 90 minutes. In a pluriparous mare, the sequence may be more rapid, with stage II completed within 30 minutes after oxytocin administration. Many veterinarians have traditionally administered oxytocin intramuscularly.[12] Much of the recent literature has reiterated the observation that the mare's response (both in speed and violence) is proportional to the dose of oxytocin given. The higher the dose of oxytocin the faster the delivery of the fetus. Oxytocin has been reportedly used to induce parturition in six mares with all foals normal and healthy within approximately 34 minutes. None of the mares had a retained placenta and all lactated normally. The dose of oxytocin ranged from 40 to 60 units. The author prefers to administer 20 units of oxytocin intramuscularly to mares with a minimum of 2 cm cervical dilation and has not had any of the reported problems described later in this chapter.

In mares with at least 2 cm cervical dilation and other signs of preparedness for delivery, the administration of 20 units of oxytocin is followed in approximately 5 to 10 minutes with restlessness and slight colicky pains, which include tail movements and looking at the flank. Within 20 minutes, the mare will be walking, frequent defecation will be occurring, the tail will be held partially up for long periods of time, frequent urinations will occur, she may repeatedly move from the standing position to the recumbent position, and sweat will appear in the area of the shoulder. The movements will become more pronounced and the sweating will extend to other areas of the body. Rupture of the chorioallantoic membrane will occur approximately 30 minutes following oxytocin administration. Delivery of the fetus through the vulva will commence in approximately 30 to 60 minutes following administration and will take approximately 30 to 40 minutes. The fetal membranes should be passed within 3 hours or the mare should receive additional oxytocin as a treatment for the retained fetal membranes.

Reportedly, oxytocin is dangerous when used to induce parturition because it has the capability to induce delivery whether the fetus is mature or not. The response

of the mare is to release larger quantities of prostaglandin than occurs during a natural parturition and is therefore a pharmacologic not a physiologic induction. Fetal membrane retention occurred as an undesirable effect in two of three mares when oxytocin was given by slow intravenous drip for longer than 3 hours. Four of five mares induced with oxytocin produced foals that were weaker than normal, and one had to be "destroyed." Cases of malposition have been reported following induction with oxytocin. It is recommended to examine the fetus 20 minutes following oxytocin administration to determine the fetal position and to correct any malposture or malposition. The author believes that all these side effects could and do occur in natural deliveries and that some of the effects may be related to dose or route of administration.

Prostaglandin F_2 alpha ($PGF_{2\alpha}$) reportedly failed to induce parturition in the mare.[13] $PGF_{2\alpha}$ can also cause very strong myometrial contractions and may decrease foal survival and increase fetal weakness and risk of death due to early placental separation.[13,14]

Fluprostenol is capable of inducing parturition in mares. Fluprostenol causes less myometrial stimulation than $PGF_{2\alpha}$ and, therefore, has been successfully used for induction. Mares with a closed cervix can be induced with fluprostenol at a dose of 2.2 μg/kg. The time to delivery should be approximately 4 hours. Fluprostenol was administered to 17 ponies, horses, and donkeys to induce parturition. The prostaglandin F metabolites (PGFM) increased after injection in mares that delivered within 90 minutes following injection and peaked during the maximal expulsive efforts of parturition. If parturition required longer than 90 minutes, the PGFM increased at various times following injection and peaked before the maximal expulsive efforts.

Thirty-three mares were induced to foal with prostalene or fenprostalene based on presence of prefoaling mammary secretion. Twenty-three mares served as control subjects and were monitored but not induced. All mares given fenprostalene delivered within 3.9 hours following injection without complication. Seventy-five percent of mares receiving prostalene delivered within 3.7 hours and the remainder delivered within 30 to 56 hours following injection without difficulty. Tests were performed to determine the content of calcium carbonate or the total "hardness" of the colostral secretion. Mares were monitored beginning 10 days prior to the expected foaling date and the secretion checked daily for 3 days and then twice daily until parturition.

Combinations of fenprostalene followed by oxytocin in 5-unit doses at 20-minute intervals has been recommended as a means to reduce the approximate 4-hour time interval following the prostaglandin analogue administration until delivery. Oxytocin was administered 1 hour following the initial injection and the delivery time was more predictable.[12]

Glucocorticoids have reportedly failed to induce parturition in the mare. Dexamethasone, 100 mg at 24-hour intervals until parturition occurs, has been utilized but with less reliability than oxytocin. The average induction time of 4 ± 1.6 days was considered to be undesirable because qualified assistance probably would not be present when required. Because suppression of the immune response is a disadvantage of corticosteroid therapy, it is thought that the use of this substance to induce parturition may be undesirable.[13] Combinations of $PGF_{2\alpha}$ and flumethasone, a synthetic glucocorticoid, have been reported to successfully induce parturition.

Dystocia

Dystocia is defined as an abnormal or difficult delivery that may or may not require assistance. A dystocia may result in injury or death to the mare or the foal or both. Dystocia occurs in fewer than 1% of equine parturitions. Certain breeds or the crossbreeding of animals of extreme size difference when the mare is smaller may increase this percentage. Although mares have fewer problems with deliveries than other domestic species, when difficulties are present they are true emergencies. The reason for this is the rapid and very strenuous nature of the delivery process in this species.

Fetal causes of dystocia account for the majority of difficult deliveries. The primary reason is postural abnormalities of the long fetal extremities. Positional and presentational abnormalities can also occur but to a lesser degree. The fetus should be expelled from the uterus within 30 minutes with a maximum of 70 minutes following the rupture of the chorioallantoic membrane. Caution should be used when assisting in the extraction of a fetus to be certain the uterus, the cervix, the vagina, the vestibule, and the vulvar lips are not lacerated by traction applied too rapidly. Extraction of a fetus in an abnormal posture or position should not be attempted until the abnormality is corrected because of the increased probability of laceration. The round shape of the mare's pelvis compared with that of the cow reduces dystocias caused by large fetuses. Other less frequently encountered fetal causes of dystocia include fetal anasarca, ascites, fetal tumor, hydrocephalic fetus, fetal monster, and mummified fetus. Maternal causes of dystocia include uterine torsion, abnormally small pelvis, uterine inertia, immaturity, constriction of the cervix or vagina, and other causes unrelated to the fetus.

The approach to a dystocia should not be hasty or heroic. A well-planned approach will be far more successful than one that overlooks major points of information critical to a successful delivery. Important aspects of this approach are a complete history and a rapid but complete physical examination. An obstetrician is often under real or perceived pressure from the owner upon arrival and may be tempted to rush the approach. The procedure will be far more successful if a reasonable time is taken for preliminary information and examination.

Notation should be made of the mare's behavior, posture, character of her breathing, ability to rise and remain standing, response to stimulation, and degree and frequency of straining; the condition of the allantoischorion; the presence of the amnion, visceral organs, or fetal extremities protruding from the vulva; and vulvar discharges and the appearance of the vulva. If possible prior to examination, the mare should be placed in a relatively dust-free, quiet area. The mare's tail should be wrapped and secured to her neck with a cord.

Next, the reproductive tract and fetus should be examined. The author prefers to perform this procedure initially via transrectal palpation. This approach permits evaluation of the uterine body more anteriorly than may be possible via the vaginal canal, thus allowing assessment of the existence of uterine torsion, rupture of uterine arteries, or uterine rupture on the dorsal surface. Fetal viability can also be assessed using the transrectal approach. The perineal area is then washed and, with the use of a sleeve and sterile lubricant, the vaginal, cervical, and uterine walls are examined for possible lacerations. This procedure should always be performed before examination of the fetus to eliminate the possibility of missing a significant lesion.

Only after the determination of the absence of lacerations should the fetus be palpated through the vaginal canal to determine its presentation, position, posture, and viability. Several reflexes can be used to determine the viability of a fetus. Depression of the eyes should initiate either movement of the fetal head or, in a depressed fetus, only movement of the eye itself. A finger inserted deeply into the mouth should stimulate the sucking reflex. With a caudal presentation, a finger can be inserted into the fetal anus to detect movement of the entire fetus or simply contraction of the anal sphincter. The skin of the fetus can be pinched or a limb can be flexed or extended maximally to stimulate a response and thus indicate fetal viability. A stethoscope on ultrasonographic examination can also be performed for detection of fetal heart beat or blood flow. The relative size of the fetus and that of the pelvis and birth canal should be approximated during this examination and identification of any abnormalities of the fetus that could compromise the delivery should be made. Following the preceding examinations, the owner should be informed as to the prognosis for the mare and fetus and the cost of the recommended procedure before therapy is initiated.

All genital examinations and obstetric procedures should be performed as sanitarily as is feasible. Obstetric procedures are easiest with the mare in the standing position with epidural anesthesia unless the mare has been anesthetized and is in dorsal recumbency. Lateral recumbency is the most difficult position. Stocks are desirable to reduce side-to-side movement of the mare if one remembers that mares may suddenly go from the standing to the sternal or laterally recumbent position, making removal from stocks difficult. Anesthetized mares can be positioned with the rear quarters higher than the head on an incline or placed in dorsal recumbency with the rear feet elevated using a chain hoist (use caution following epidural). Elevation of the hindquarters permits the mare's abdominal viscera to move away from the pelvic area. The uterus and fetus will also move cranially, permitting more space to manipulate the fetus.

Fetal presentation refers to the relationship of the spinal axis of the dam. Cranial longitudinal presentation is where the fetal head is presented first at the vulva. Caudal presentation occurs when the rear limbs or pelvis are presented first. Transverse and vertical presentations occur when the mid portion of the fetus is the first portion to contact the cervix. Position is defined as the relationship of the dorsum of the fetus to the quadrant of the maternal pelvis. The possibilities that exist in longitudinal presentation are dorsosacral, right dorsoileal, dorsopubic, and left dorsoileal. Posture refers to the relationship of the fetal extremities to the fetal trunk. A normal delivery is defined as cranial longitudinal presentation, dorsosacral position, with the fetal head, neck, and forelimbs extended.

Chemical restraint is often necessary to facilitate obstetric manipulations in the mare. Owing to their potent cardiovascular effects, sedatives (xylazine, detomidine) and tranquilizers (acepromazine) must be used with caution in compromised patients. Prior to administration of any sedative or tranquilizer drugs the cardiopulmonary status of the mare should be evaluated and doses adjusted accordingly. The potential for subsequent general anesthesia should always be considered when administering sedative/tranquilizer drugs to the equine dystocia patient.

Administering a combination of xylazine (0.66–1.1 mg/kg IV) and acepromazine (0.04–0.06 mg/kg IV) generally provides a reasonable degree of patient cooperation and a reduction in the severity of uterine contractions. This combination should provide 20 to 40 minutes of relief and can be repeated. Butorphanol (0.01–0.015 mg/kg IV) may be added to improve the efficacy of the preceding combination. In some cases general anesthesia may be required to facilitate fetal repositioning and removal. When available, inhalation anesthesia is always preferred for obstetric work.

Intravenous general anesthesia can be safely performed in the field setting. The practitioner should review a contemporary veterinary anesthesia text for a more thorough understanding of the procedures involved and the potential risks of equine field anesthesia. Sedation with xylazine (1.1 mg/kg IV) is followed by induction with a combination of diazepam (0.06–0.1 mg/kg IV) and ketamine (2.2 mg/kg IV). This combination should provide approximately 10 to 15 minutes of anesthesia when used alone. The duration of anesthesia can be extended with administration of "triple drip" (also called GKX), which is made by adding 1 mg/ml of ketamine and 0.5 mg/ml of xylazine to 5% guaifenesin solution. Triple drip is administered at a rate of 0.08 to 1.6 ml/kg/hour IV (or 1–2 drops/sec/450 kg). The 1 drop/sec rate will generally extend recumbency, but will often not cover significant surgical stimulation later in the procedure. The 2 drops/sec rate will generally extend complete surgical anesthesia for the duration of the procedure. An infusion rate of 3 drops/sec has been reportedly used without adverse effects. A more stable plane of anesthesia will result if triple drip administration is started immediately following anesthetic induction. If field anesthesia must be extended beyond 1 hour, the second bag of triple drip should be made using half the concentration of xylazine to minimize the accumulation of this drug. Recovery from triple drip is generally very smooth and usually takes 15 to 30 minutes following short anesthetic periods. Expect recovery time to increase as anesthetic duration is extended. α_2-Adrenergic antagonists (yohimbine, tolazoline) may be used to reverse some of the xylazine sedation to shorten these longer recovery times. They should be administered in small increments (0.005 mg/kg for

yohimbine and 0.5 mg/kg for tolazoline) and not until the horse has been in sternal recumbency for some time to minimize the risk of a rough recovery. It is very important to be patient when using smaller doses of reversal agents to produce a titrated response. For best results doses should be given at 3- to 5-minute intervals.

Repeated bolus administration of xylazine-ketamine can be used as an alternative to triple drip for extending the duration of equine field anesthesia. One third to one half of the initial dose of each drug is administered intravenously as a bolus when signs of a lightened plane of anesthesia are observed. Xylazine dose should be systemically reduced when numerous boluses are required. The anesthetic plane achieved using this approach is not an even plane and requires more attention than when using triple drip.[15]

One of the challenging aspects of treating animals in dystocia is that there are only a limited number of methods available for correction. The most difficult decision is to select the method that will achieve the objective of delivering a fetus alive with minimal trauma to the mare and the fetus. The second most important aspect of successful management of dystocia is to have access to the mare early in the delivery process. The possibilities for dystocia correction include mutation, forced extraction, fetotomy, laparotomy, and laparohysterotomy. Although euthanasia of the mare may be selected to terminate the case because of economics or the physical condition of the mare at the time of arrival, it is not included as a treatment for dystocia as it does not fulfill the definition of mare and foal survivability.

The amount of assistance necessary depends on the technique to be employed for correction. The minimal amount of assistance recommended for an obstetric procedure is two persons. One person is at the head of the mare for the entire procedure and one assists the obstetrician with equipment and with traction or fetotomy cuts when required. Generally three or four assistants make the procedure go more rapidly.

Obstetric equipment recommended by the author includes three obstetric chains (60 inch), three obstetric chain handles, fetatome (Utrecht or flat tube model), a Krey-Schottler hook, a wire introducer, two wire handles, a detorsion rod, a fetotomy finger knife, a rope snare, obstetric wire, a Kühn crutch, rubber sleeves, buckets, a stomach pump, a nasogastric tube, and lubricant. The lubricant of choice is polyethylene polymer (J-Lube*), which comes in powder form and is water soluble. It can be mixed at the site and delivered into the uterus with a stomach pump and tube. Although not all of this equipment is essential for every dystocia, its availability will improve the quality of the obstetric manipulation and reduce the work required to obtain desirable results.

Mutation

The most frequently used method of correction is mutation. Mutation is the procedure used to correct malposture, position, and presentation followed by delivery of the entire fetus. This method should be the least traumatic and should be employed prior to the use of forced extraction. Mutation may not always be possible or indicated, depending on the length of time the mare has been in labor, viability of the fetus, and the cause of the dystocia.

Mutation involves returning the fetus to a normal presentation, position, and posture by repulsion, rotation, version, and adjustment or extension of the fetal extremities. It is primarily indicated when the fetus is alive and the procedure can be performed in a relatively short period with a high probability of success. It has been found by the author that in the case of a dead fetus, fetotomy procedures to eliminate a postural abnormality are faster and less stressful to the mare and the obstetrician than attempts to mutate unless the dystocia has been of minimal duration and sufficient area exists within the uterus to permit manipulation. Repulsion of the fetus into the abdominal cavity away from the pelvis aids in creating more space in which to perform obstetric procedures. Lubrication is very important with all obstetric procedures, including mutation, and may make the difference between success and failure of the technique. Extension of a flexed limb is accomplished by repelling the proximal portion while the midportion of the limb (carpus or tarsus) is rotated laterally (Fig. 14-5). Following this, the foot is brought medially and extended into the pelvis. If correction is not possible in 15 to 20 minutes, another method of correction should be selected.

Lateral deviation of the head is a common cause of dystocia because of the relatively long neck of the fetus (Fig. 14-6). The fetus should never be delivered with this postural abnormality because of the potential for injury to the birth canal. The operator's fingers can be inserted into the fetal mouth and traction applied to bring the head toward the brim of the pelvis. Much of the success of this technique depends on the amount of space within the pelvic area. In cases of prolonged labor, there may be no available space for the extension of the neck. The limb on the side opposite the deviation of the neck may need

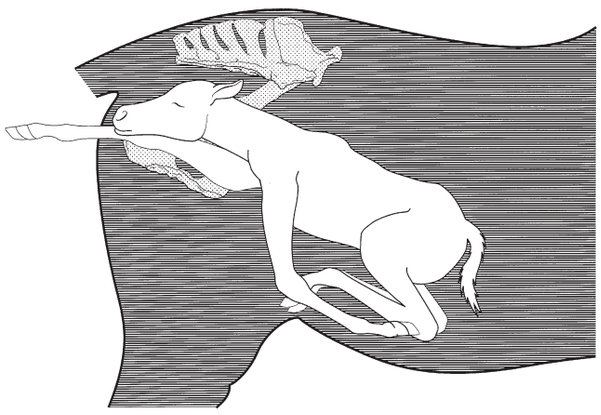

Fig. 14-5 Anterior presentation, dorsosacral position, with the head and right forelimb extended and left shoulder flexed. (From Threlfall WR: *Threlfall's equine obstetrics.* Powell, OH: Global Reproductive Services, 1996.)

*Jorgensen Laboratories, Loveland, CO.

to be placed into carpal or shoulder flexion to permit sufficient space for extension of the head. Attachment of a rope snare to the fetlock area of this limb facilitates postural correction following extension of the neck. A repulsion rod can be used to repel the fetus by placing it in the area of the sternum and pushing gently. The muzzle can be brought into the pelvis by pushing the poll of the head to the opposite side of the pelvis while applying traction to the mouth or muzzle (Fig. 14-7). A rope snare can also be used to facilitate this technique. As traction is applied to the rope snare, the hand is used to guide the neck and head into the pelvic canal. A rope snare placed on the mandible can be used as a method of postural correction but should never be used as a point of traction because the mandible fractures easily and the incisors can be removed in such attempts. If the fetus is alive, anesthetizing the mare and placing her in dorsal recumbency with the rear end elevated early in the correction process may be beneficial. If the fetus is dead, an immediate fetotomy may be the preferred method of correction.

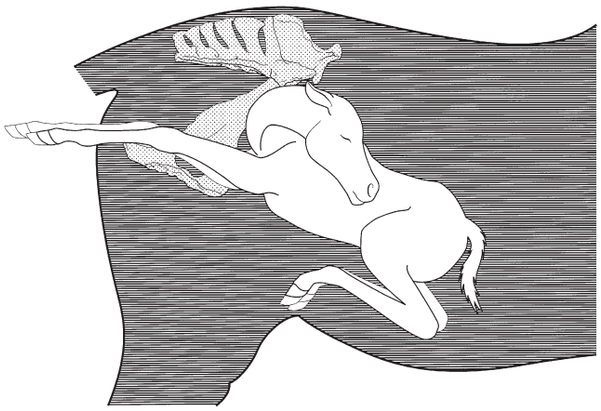

Fig. 14-6 Left lateral deviation of head and neck with extended forelimbs and fetus in anterior longitudinal presentation with dorsosacral position (lateral view). (From Threlfall WR: *Threlfall's equine obstetrics.* Powell, OH: Global Reproductive Services, 1996.)

Cranial longitudinal presentation, dorsosacral position with head, neck, and forelimbs extended and with bilateral hip flexion can be diagnosed following failure of delivery of a fetus that appears to be in proper presentation, position, and posture (Fig. 14-8). Determination is made on vaginal examination that the fetal rear hooves are located in the mare's pelvis near the elbows of the fetus. The first step in correcting this abnormal posture is to repel the rear hooves over the brim of the pelvis and into the abdominal cavity. This may not be possible owing to forced extraction applied before an accurate diagnosis was made or to the time elapsed waiting for delivery of the fetus in what appeared to be a normal presentation, position, and posture. A dead fetus may have to be transected in the lumbar area and, if possible, repelled or the pelvis bisected and removed. If the fetus is alive, it may be impossible to deliver a viable offspring even if the mare's rear quarters are elevated or by cesarean section.

Correction of carpal flexion (Fig. 14-9) by mutation in live fetuses is best accomplished by grasping the limb in the area of the metacarpus with the hand positioned so the thumb and fingers completely encircle the limb. The proximal portion of the limb is then repelled into the uterus. The limb is rotated with the carpus pushed laterally and the hoof moved medially under the neck of the fetus and then pulled cranially with respect to the fetus. Once the hoof can be brought into the pelvis, the limb is extended. If this technique is not possible with the hand alone, a rope snare can be attached to the wire introducer and placed around the flexed carpus and formed into a loop. The loop is moved distally on the limb until the snare is distal to the fetlock if possible. The rope is tightened and traction is applied as the proximal portion of the limb is repelled. The disadvantage of this technique is the loss of control of the direction of the

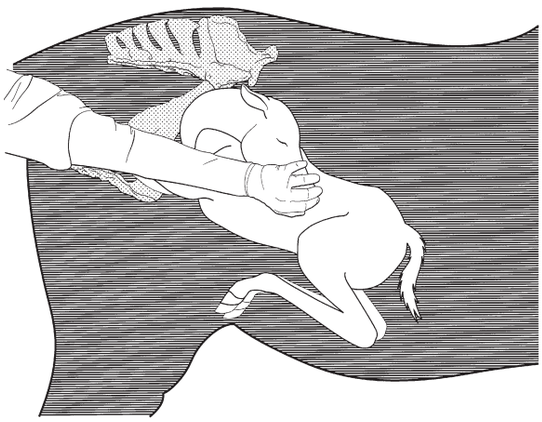

Fig. 14-7 Correction of head and neck lateral deviation by hand alone. Hand is placed around muzzle or fingers are placed in lateral commissure of mouth and a pulling pressure is applied. (From Threlfall WR: *Threlfall's equine obstetrics.* Powell, OH: Global Reproductive Services, 1996.)

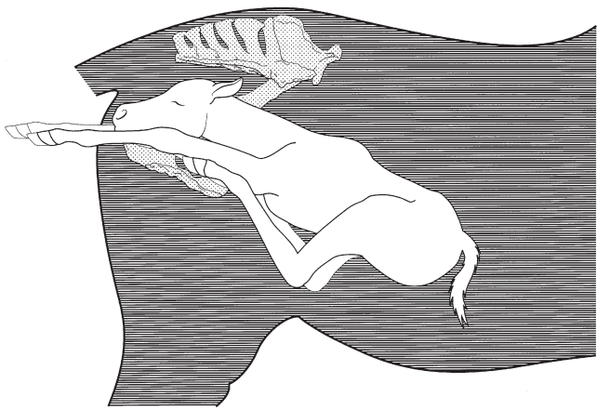

Fig. 14-8 Anterior presentation, dorsosacral position with head, front limbs, and rear limbs extended. (From Threlfall WR: *Threlfall's equine obstetrics.* Powell, OH: Global Reproductive Services, 1996.)

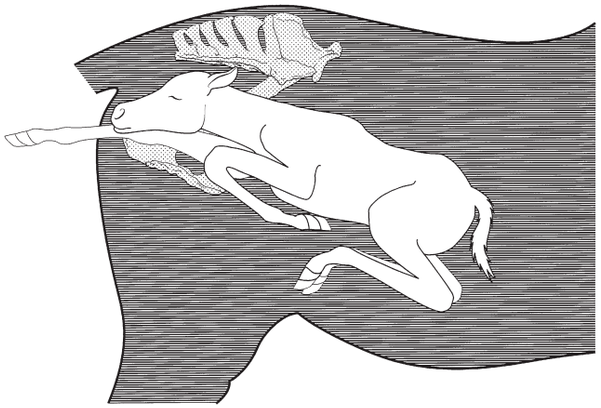

Fig. 14-9 Anterior presentation, left carpal flexion posture. The shoulder is converted to a carpal and elbow flexion. (From Threlfall WR: *Threlfall's equine obstetrics.* Powell, OH: Global Reproductive Services, 1996.)

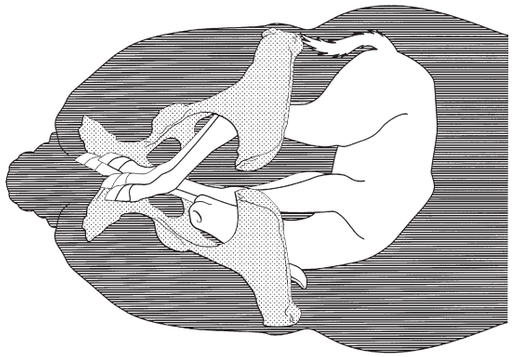

Fig. 14-10 Ventral transverse presentation, right cephaloileal position with head and limbs extended. (From Threlfall WR: *Threlfall's equine obstetrics.* Powell, OH: Global Reproductive Services, 1996.)

hoof as it clears the brim of the pelvis. However, it is an acceptable and beneficial technique that has application in some dystocias with a live fetus.

Dorsoilial and dorsopubic positions should be corrected before delivery of the fetus to ensure minimal trauma to the birth canal during extraction. Although other methods to rotate the fetus have been described, such as the hand alone to push or pull the fetus in the desired direction, these techniques require more physical work and are not as successful as the use of a detorsion rod. Although techniques have been described using chains without a detorsion rod for correction, there is a higher probability of injury to the vagina of the mare and the limbs of the fetus with this technique.

After determining the viability of a fetus in transverse presentation (Fig. 14-10), the method of correction should be employed immediately. If the fetus is alive, the best possible choices would be to do a cesarean section or attempt to elevate the rear quarters of the mare to provide additional space for manipulation. If possible, an attempt should be made to deliver the fetus in caudal presentation; the reason for this is to eliminate the need to direct the fetal head, and this may make the delivery more rapid. If the fetus is dead, transection of the fetus should be attempted if sufficient space is available. A major problem associated with a transverse presentation is that affected mares have usually been in labor for a much longer period of time than believed by the owner, since there may be no cervical pressure to induce the more visible expulsive efforts of stage II labor.

Forced Extraction

Forced extraction is generally combined with mutation or fetotomy. Forced extraction is not intended to replace other techniques but to aid in the delivery process. If used unwisely, forced extraction results in injury to the fetus or the mare's reproductive tract or both. Extractive force is used with uterine inertia, following corrective mutation, with inadequate cervical dilation, in caudal presentations and in combination with fetotomy. Extreme traction should never be applied in lieu of corrective mutation, a fetotomy or cesarean section because of the possibility of trauma to the mare or fetus. The position and posture of the foal should be as near to normal as possible before traction is applied. Traction placed on the fetus should be slightly dorsal and caudal until the forelimbs, head, and neck are at the vulvar lips. The direction of the traction is then directed more ventrally as more of the fetus is visible. Traction should be directed mostly ventral by the time the shoulders have passed through the vulvar lips.

When the operator is experienced and uses good judgment, forced extraction is relatively safe. It can cause various injuries, however, including damage to the head, limbs, and spinal cord of the fetus; damage to the uterus and cervix and adjacent structures; eversion of the uterus; and lacerations of the vagina, vulva, perineal body, and rectal floor. Aftercare following forced extraction may include uterine stimulants placed into the uterus or administered systemically. The use of antibiotics may be indicated to reduce the number of organisms that were introduced during the procedure. Broad-spectrum antibiotics are generally recommended for this purpose.

If it becomes apparent that forced extraction is not going to be successful, another technique for correction should be attempted. A common mistake made by obstetricians is to continue using a technique beyond a reasonable time in anticipation that eventually it will be successful. Determining the time to abandon one technique for another is gained through experience and is always a challenge.

Fetotomy

Fetotomy is indicated in many instances because the manipulative time and trauma to the mare can be reduced over that of mutation and forced extraction if the fetus is dead.[16] Fetotomy is a reduction in fetal size by removal of the extremities or dissection of the body. Specific indications include the presence of a dead fetus in the absence of excessive uterine contracture; a fetus that

is not overly emphysematous; abnormalities in fetal presentation, position, or posture; the presence of an oversized fetus in relationship to the maternal birth canal; the presence of fetal abnormalities such as ankylosis of joints; and incomplete cervical dilatation. The decision to perform a fetotomy also depends on the veterinarian's opinion that there is sufficient space in the dam's genital tract to perform the procedure and on the experience of the obstetrician. The advantages of a fetotomy include reduction of fetal size, elimination of need for a cesarean section, minimal assistance necessary, decreased trauma to the mare, less infertility than following cesarean section, less aftercare than with cesarean section, shorter recovery time than with cesarean section, greater practicality as a "field" technique than a cesarean section and greater monetary economy to both the owner and the veterinarian. A cesarean section is the preferred approach by many veterinarians over that of fetotomy in cases of a live or dead fetus that cannot be removed by mutation and forced extraction. Reasons given for not using fetotomy include overstretching of the cervix; more physical exertion by the veterinarian; lacerations of the uterus, cervix, vagina, and of the operator; and the procedure being "too hard on the mare." A cesarean section, however, cannot generally be performed under farm situations where no technical assistance or facilities are available, whereas fetotomies can be performed under these conditions. Supposedly to reduce severe injury to the genital tract, it has not been recommended to perform fetotomies that require more than one or two cuts.[17] However, the duration of the fetotomy, the damage to the reproductive tract prior to the initiation of the fetotomy, and the correctness of the cuts have a major influence on the success of the procedure. The other consideration on the farm is what other options are available that will save the life of the mare. Also, most equine dystocias are caused by simple postural anomalies, so only one or two cuts are necessary to permit delivery of the remainder of the fetus following fetotomy in most instances. Also, fetotomy procedures are generally less costly to the owner since aftercare is considerably less and future reproductive capability is better than following a nonelective cesarean section.

One of the primary determining factors in most but not all fetotomies is the quality and type of instrumentation available to the obstetrician and familiarity of the obstetrician with the procedures. Some veterinarians consider fetotomy as the last resort, and such thinking may result in failure. If performed properly, fetotomy requires less aftercare than a cesarean section and there is less probability of systemic infection, parametritis, perimetritis; higher reproductive capability; reduction in laminitis; and a shorter time to rebreeding.

Indications for total fetotomy should be recognized early so that cesarean section can be considered before a large amount of effort and time is expended on fetotomy. When performing a complete fetotomy on a fetus in cranial presentation, the following sequential cuts with some variations are recommended: amputation of the head; amputation of a forelimb; amputation of the opposite forelimb; transverse section through the third thoracic vertebra and removal of the remaining neck and the thorax cranial to the cut; transverse cut through approximately the third lumbar vertebra and longitudinal transection of the thorax; and finally a longitudinal cut through the pelvis. A partial fetotomy with one or more cuts, evisceration, and lubrication may enable simple mutation or forced extraction and delivery of the remainder of the fetus.

Dismembering a fetus in cranial presentation may be indicated for various reasons. Amputating a protruding fetal head in dystocias in which the forelimbs are retained provides more space for repositioning or removing the limbs. Owing to the rapid normal delivery of the fetus in the mare, partial placental separation and death of the fetus frequently occur by the time the obstetrician arrives at the dystocia. This situation plus the available assistance and surroundings may make fetotomy the only method possible to correct the dystocia and save the mare. An extended head is separated through the atlanto-occipital junction with the fetatome head resting against the caudal border of the ramus of the mandible. To do this, a loop of wire that has been threaded through the fetatome is placed around the fetal head and behind the ears in the area of the axis. After proper lubrication, it may be possible to deliver the remainder of the fetus by traction following head removal. The remaining portion of the fetus is within reach; it cannot drop further into the uterus because of pressure against the fetus. It is imperative that following placement of the obstetric wire, the position of the wire be checked one last time before any attempts to dissect the fetus are made. The hand of the obstetrician always covers the head of the fetatome during the amputation procedure. The person doing the sawing must fully understand that he or she is to start only when instructed to do so and to stop immediately when instructed to do so.

Probably the most common indication for amputating the fetal head and neck is lateral deviation. The forelimbs may have to be repelled if possible to provide more space in the birth canal for this fetotomy procedure. If the limbs cannot be repelled with little effort, the limb opposite the direction of the head deviation or opposite to the limb in flexure should be removed to provide additional working area for removal of the head. Neck amputation involves inserting the wire introducer with the obstetric wire attached around the fetal neck and then withdrawing it from the birth canal. The wire is threaded through the fetatome and the head of the fetatome is held tightly against the fetal neck at its greatest curvature. Following this cut, the head and attached portion of the neck of the fetus can be removed with the use of a Krey-Schottler hook.

Another common cause of dystocia in cranial presentation is a flexed forelimb. The limb is usually flexed at the shoulder and extends beneath the fetal body. A wire introducer with wire attached is passed dorsally over the shoulder and between the fetal trunk and the limb. The wire is then grasped ventrally and pulled outside of the genital tract. The fetatome is inserted into the uterus so that the head is approximately 5 cm dorsal and medial to the scapula's spinous process. After the limb has been severed, it is removed. Following amputation of the limb, the area of the scapular attachment is examined for

remnants of cartilage or bone fragments. If these are found, they can be removed with the fingers alone using blunt dissection or with the aid of the finger knife. The fetus can be removed if the flexed limb was the only cause for the dystocia, the opposite limb can be removed in the same manner, and the remaining cervical vertebral bodies used to secure the Krey-Schottler hook. In this instance the single point of traction is sufficient for delivery. If there exists a flexed carpal or, in the case of caudal presentation, tarsal joint, a cut distal to the joint may alleviate the dystocia with a dead fetus and save the mare further discomfort and injury that may occur with attempts to mutate the limb. The limb is amputated by placing obstetric wire around the limb with the wire introducer. The wire is then threaded through the fetatome and the cut is made distal to the joint. This permits the attachment of an obstetric chain to the remaining stump in order for this limb to be used as a point of traction.

A complete fetotomy performed in caudal presentation includes the following cuts: The first may involve either of the rear limbs depending on access. The second cut removes the opposite hind limb. The third cut removes the caudal aspect of the fetus including the remaining pelvis and the majority of the lumbar vertebra. The next cut is made through the area of thoracic vertebra immediately caudal to the scapula. Then the forelimbs can be removed one at a time. The neck and head with the remaining thorax could be removed together or separately. Other modified cuts may be necessary in cases such as hydrocephalus, when the head must be sectioned prior to removal, or in instances in which limbs may need to be sectioned, such as with a tarsal flexion, before delivery is possible.

With a true breech presentation only the tail is in the birth canal and may be observed protruding from the vulva. Both hind limbs are flexed and retained beneath the fetal body. A wire introducer is inserted dorsally over one hind limb between the trunk and the limb, and a hand is slipped under the limb to grasp the wire introducer ventrally and pull the end of the obstetric wire from the birth canal so that it can be threaded through the fetatome. The head of the fetatome is placed adjacent to the tailhead and a flexed hind limb is removed with a cut approximately through the coxofemoral joint. The amputated limb can then be removed. It usually is more rapid and less exhausting for the surgeon to remove the opposite retained limb in a similar manner rather than trying to mutate it. A Krey-Schottler hook can be inserted into the fetal pelvis to serve as a point of traction and deliver the remainder of the fetus after adequate lubrication.

In the case of an extended rear limb amputation, following placement of an obstetric chain on the rear limb with a loop above and another loop below the fetlock, the fetatome threaded with a loop of wire is placed as was described for removal of an extended forelimb. The loop of wire is fixed adjacent to the third phalanx alongside the chain. The head of the fetatome is moved slowly proximally toward the body of the fetus lateral to the limb until the head is 5 cm dorsal and cranial to the tuber coxae and is fixed in this position by attaching the chain to the fetatome. The wire is then moved alongside the medial surface of the limb while maintaining tension on the wire to prevent kinking or twisting. Immediately prior to dissection, the wire is checked to be certain of its placement. If indicated, the opposite rear limb can be removed in a similar manner. Once the limb or limbs have been removed, the fetal abdominal cavity should be incised and the abdominal contents removed to reduce the size of the abdomen. Removal of one or both rear limbs is a fairly common fetotomy procedure employed to permit the removal of an equine fetus due to abnormal posture of the rear limbs. If it is determined that the remainder of the fetus is still too large for forced extraction, a cut can be made through the area of the second lumbar vertebra to remove the pelvic portion of the fetus. If this problem was foreseen prior to removal of the second rear limb, the pelvis could be cut diagonally, thus allowing removal of the remaining rear limb and a portion of the pelvis with one cut. Fetotomy aftercare generally includes the administration of systemic uterine stimulants such as oxytocin and the infusion of antibiotics or antiseptics into the uterus.

Laparotomy is indicated primarily in those cases involving uterine torsion in which other methods of correction via the vagina were unsuccessful. The torsion may be evident months prior to the onset of labor as determined by prolonged colic-like signs that fail to respond to therapy for gastrointestinal pain. The diagnosis can be easily made by transrectal palpation of the uterus and broad ligaments. The finding of one broad ligament traversing the midline to the opposite side dorsal to the uterus with the other broad ligament nonpalpable is sufficient information to make the diagnosis. The correction of the uterine torsion via laparotomy can be achieved by one of several abdominal approaches with the mare standing or recumbent. The author prefers the standing flank approach on the side of torsion origin. For example, a counterclockwise torsion would be approached from the right flank. The author prefers this approach because the uterine horn can be pulled or pushed into normal position.

Laparohysterotomy

Laparohysterotomy is another option for the alleviation of dystocia. Cesarean section refers to delivery of a fetus through incisions in the abdominal wall and uterus of the dam. Cesarean section has its best advantage following repositioning of a uterine torsion or when preplanned and the time and facilities are immediately available to permit delivery of the fetus while alive. Indications for cesarean section include a previously fractured pelvis or other obstruction that reduces pelvic diameter; a small pelvis; an extremely large fetus; abdominal wall or prepubic tendon ruptures; uterine torsion that is not correctable by a vaginal approach or by rolling the mare; malpresentations that are not correctable by other methods; emphysematous fetuses; fetal anomalies; and a live fetus that cannot be delivered by any other way. One complication of cesarean section is rupture of the uterine artery during manipulation. The biggest problems encountered in regard to a cesarean section are mare and foal survival and future reproductive performance of the

mare. An elective cesarean section gives the best prognosis for both.

The considerations involved in selecting a site for a cesarean section include the operative conditions and disposition of the animal. The surgeon's previous experience with operative sites is another major consideration. The possible operative sites include right and left flank with the animal standing using sedation and local anesthesia. This approach, however, is seldom recommended owing to reduced exposure of the uterus, visible abdominal wall scarring, and possible movement of the mare during surgery. The flank approaches under general anesthesia are also options, but the reduced uterine exposure and visible scar following surgery are disadvantages. The primary advantage of the left flank over the right is that the cecum is less of a problem and thus does not obstruct access to the uterus. However, intestines may make the left flank approach difficult. Possible operative sites in a recumbent animal include low approaches through either flank, the paramedian area, or the abdominal midline. The ventral midline incision is most commonly used and has the advantage of providing excellent exposure of the uterus. There is very little hemorrhage when this site is used. There may be one or two large veins in this area that form an anastomosis from the right to left subcutaneous abdominal veins. These veins, if present, should be ligated prior to making the incision in the subcutaneous tissue.

After the fetus is removed through the incision, the placenta is removed if it is free within the uterine cavity. If the placenta is still adhered to the endometrium, it is left in the uterus and an antibiotic solution or boluses are placed into the lumen before closure. If hemorrhage occurs from the subendometrial veins, the veins are ligated independently or a ligature is placed in the area of the hemorrhage, including endometrium, subendometrium, and myometrium. Simple continuous, continuous interlocking, or similar suture patterns can also be used to control hemorrhage. A Cushing suture pattern can be used to infold the incision site. The suture should be placed so that very little if any suture material is apparent on the serosal surface following closure. This will reduce the adhesions that form between the uterine wall and abdominal viscera. The care of mares following a cesarean section should be provided on an individual basis because it is not feasible to treat all animals the same way and achieve good results. Repeated administration of oxytocin is advisable to enhance uterine involution.

Following the correction of dystocia by any method, the uterus should be examined to ensure that another fetus is not present. The tone of the uterine musculature should also be assessed. The birth canal should be palpated for evidence of lacerations.

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CHAPTER 15

Abnormalities of Lactation

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Lactational abnormalities are rarely encountered in mares. Yet when a particular mare is involved and her foal is at risk, these problems are highly significant to the mare's owner. Equine lactational problems may have received so little study or documentation in the veterinary literature because they are often easily corrected and because adequate substitutes for mare's milk are available. Abnormalities of lactation can be classified as functional or infectious. Functional abnormalities include agalactia, galactorrhea, precocious lactation, premature lactation, and eclampsia (lactational tetany). Mastitis is the main infectious abnormality.

To understand these abnormalities, it is important to comprehend lactational physiology and anatomy of the equine mammary gland. These topics have been reviewed.[1]

ANATOMY AND PHYSIOLOGY

The equine udder consists of two flattened, cone-shaped glands, each terminating in a short, laterally flattened teat. Each gland contains distinct lactiferous duct systems, organized into lobes and lobules that are incompletely separated from one another by fascial trabeculae extending into the gland from the medial and lateral suspensory ligaments. The cranial lactiferous duct system is the largest. Each teat has two or three orifices, each connecting to its own lactiferous duct system within the gland. The orifice leads to a streak canal that connects to a cistern within the teat. Each teat cistern opens into a gland cistern above the teat in the lactogenic portion of the gland.[2,3] The small size of the teat orifices and the separate lactiferous duct systems associated with each gland complicate intramammary therapy in cases of mastitis.

During the first gestation the mare's udder changes from two small inguinal skin folds with teats into recognizable mammary glands under the influence of the gestational hormones. Estrogen promotes mammary gland duct development; progesterone facilitates proliferation of the lactogenic epithelial cells and organization of clumps of these cells into mammary alveoli.

The decline in estrogen and progesterone late in gestation is associated with increased prolactin release from the anterior pituitary during the last week of gestation.[1,4,5] It is thought that prolactin release is made possible by suppression of prolactin-inhibiting factor from the hypothalamus. Dopamine is likely the prolactin-inhibiting factor in mares and other animals.[1] Thyrotropin-releasing hormone (TRH) may function as a prolactin-releasing factor.[4] A rise in prolactin is associated with final development of the mammary gland and the onset of lactogenesis. In addition to prolactin, adrenal corticosteroids, insulin, growth hormone, glucagon, and thyroid hormones play a role in mammary gland development and lactogenesis, although their exact functions in mares are poorly understood.[4]

During nursing, milk ejection occurs as myoepithelial cells surrounding the mammary alveoli contract in response to oxytocin released from the posterior pituitary. Oxytocin is synthesized in the hypothalamus and stored in secretory vesicles within the hypothalamic oxytocin neurons. It is released from nerve terminals in the posterior pituitary. Hypothalamic oxytocin neurons are also stimulated to release oxytocin in response to vaginal stimulation, which occurs during parturition.[5]

Abnormal endocrine stimulation may lead to functional lactational abnormalities such as agalactia, hypogalactia, galactorrhea, precocious lactation, premature lactation, or eclampsia.

AGALACTIA

Agalactia must be differentiated from failure of milk letdown. The udder is slack and not engorged with milk in agalactic mares. On the other hand, some maiden mares with tender edematous mammary glands or nervous mares are reluctant to relax and allow nursing and thus retain milk in the glandular alveoli. Phenothiazine tranquilizers and warm towel massages of the udder are helpful in these mares.[6,7] In addition to their calming effect, phenothiazine tranquilizers stimulate release of prolactin and may augment lactation. Oxytocin (10–20 units) has also been used to help stimulate milk letdown once mares are relaxed.[8]

Agalactia at parturition may actually be delayed onset of lactation. This condition has been described in some maiden and multiparous mares that had small udders and only honey-like mammary secretions.[6] Many of these mares start lactating within a day or two but some may have decreased milk output. Phenothiazine tranquilizers, udder massage with hot packs, and repeated stripping of secretions from the udder may help speed the onset of lactation. TRH (2.0 mg SC, twice a day for 5 days) has been used successfully in some affected mares.[9]

Agalactia in mares without udder development is typical of fescue toxicity.[6,7,10] Fescue toxicity occurs frequently when pregnant mares are fed fescue infected with the endophyte fungus *Acremonium coenophialum*. This fungus produces a toxin (thought to be an ergot alkaloid) that suppresses prolactin release.[11] Prolactin levels return to normal by 2 to 3 weeks after the toxin is removed.[11] The endophyte is spread through fescue seeds.

A similar syndrome is seen in mares that ingest *Claviceps purpura* (ergot).[12] Fescue toxicity is most significant during the last 90 days of gestation in mares. In addition to agalactia, affected mares may experience prolonged gestation and thickened placentas, which are associated with neonatal asphyxia. Mares grazing infected fescue may also experience reduced fertility.[13] The mares experiencing prolonged gestation frequently have a history of udder development at their expected due date, but the udder involutes and they are agalactic by the time of delivery.

Treatment of fescue-induced agalactia has been aimed at overcoming suppression of the release of prolactin and possibly TRH. TRH, as well as phenothiazine tranquilizers, has been used as described in some cases.[14] More recently, the use of the dopamine receptor antagonist perphenazine (0.3 mg/kg PO twice a day) has been popular for treating fescue-related agalactia.[15] However, some mares experience severe side effects when treated with this drug, including sweating, colic, hyperesthesia, ataxia, and posterior paresis.[16,17] The dose of perphenazine should be calculated based on the patient's weight, and treatment must be carefully monitored and suspended if adverse effects become evident. The selective DA_2 dopamine receptor antagonist domperidone (1.1 mg/kg PO once a day) prevents the signs of fescue toxicosis without adverse side effects.[17]

Prevention of fescue toxicosis rather than treatment should be the primary objective. The current recommendations include the following: (1) maintain mares on nonfescue pasture when practical; (2) dilute the toxin by overseeding pastures with a palatable legume every 2 years so that 20% of the forage is a legume; (3) kill the infected fescue with herbicides and tillage and reseed with noninfected endophyte-resistant strains of fescue that are not allowed to develop seed heads for at least 2 years; and (4) remove mares from fescue during the last 90 days of gestation and provide nonfescue forage or hay.[7] Prevention of toxicosis may also improve fertility, neonatal survival, and foal growth in addition to eliminating agalactia.

Agalactia may be encountered in mares that have established lactation but prematurely cease milk production. Insufficient energy intake to support lactation must be considered, especially in mares that have experienced excessive weight loss during lactation. If the ration is adequate, the stress of pain or disease must be ruled out as the cause of cessation of lactation. Attempts to reinitiate lactation are unsatisfactory.[6]

INDUCTION OF LACTATION

Lactation may be induced in nonparturient mares that have foaled in previous years. This offers the opportunity to produce nurse mares for orphaned foals. However, the immunoglobulin levels provided by milk from an induced lactation are generally inadequate to achieve successful passive transfer in the foal. There is considerable mare variation in the production of IgG.[18,19] Sulpiride, a dopamine D_2 antagonist, successfully induced lactation in cycling mares but not in ovariectomized mares. Prolactin levels did not increase as much in the ovariectomized mares.[20] Progesterone and estrogen are required for lactation induction and the variability of these hormone levels seems to cause the variability of the amount of milk production.[21] The protocol for lactation induction includes altrenogest (44 mg/day PO), estradiol benzoate (10 mg/day IM), and sulpiride, 1 mg/kg IM, twice a day for 7 days with milking to commence on the seventh day.[18] Reinstituting the administration of sulpiride on day 23 of treatment yielded a 75% increase in milk production at day 30 as compared to an 11% increase in the group that did not receive additional treatment after day 14.[22] There is an initial difference in weight gain for the first 4 weeks between foals nursing mares with an induced lactation but by the time of weaning there was no difference in weight.[18]

HYPOGALACTIA

Hypogalactia, low milk production, may be the result of malnutrition in mares. Lactating mares require 1.7 and 1.5 times as much energy during early and late lactation, respectively, compared with nonlactating mares.[1,23] A sound feeding program is necessary to maintain adequate lactation. The use of body condition scores is a valuable method for determining adequacy of the ration to maintain lactation. Other factors that have been incriminated as causes of hypolactation include restricted water intake, selenium deficiency, and stress.[1]

GALACTORRHEA

Precocious lactation in weanling or yearling fillies and nonpregnant or maiden mares occurs occasionally for unknown reasons.[1] The cause is unknown, so treatment has been empirical and includes prevention of nursing by other animals and restriction of feed and water. Theoretically, this condition may be associated with elevated prolactin levels and thus suppression of prolactin secretion may be helpful.

Premature lactation during middle to late gestation may indicate problems with pregnancy. Impending abortion, death of one twin, placental separation, and placentitis have been associated with this condition; however, premature lactation may occur in the absence of any of these conditions. Although treatment with supplemental progesterone has been attempted in affected mares, its efficacy is unknown. There is no specific treatment.[1] Administration of the semisynthetic ergot alkaloid bromocriptine to suppress prolactin in affected mares is not indicated because it may depress progesterone concentrations during late gestation and lead to placental thickening, dystocia, and reduced neonatal survival.[15] Premature lactation during pregnancy probably has no effect on postpartum lactation with the exception of loss of colostrum in periparturient mares.[24]

LACTATIONAL TETANY

Eclampsia or lactational tetany of mares may be more appropriately discussed as a metabolic disease resulting in hypocalcemia than as a functional disease of lactation.

This rather uncommon condition is frequently associated with early lactation in mares on lush pasture that produce large quantities of milk. It occurs most commonly in the first 2 weeks of lactation, often during foal heat, but may also occur after weaning. It may or may not be accompanied by hypomagnesemia and in some cases may be associated with hypermagnesemia.[25] Eclampsia may be precipitated by heavy work or prolonged transport. Hypomagnesemia with hypocalcemia is usually precipitated by transport.[25]

Clinical signs are those typical of hypocalcemia in other species and include excitability, mild muscle fasciculations and gait stiffness, flared nostrils, profuse sweating, muscle paresis, tachycardia, synchronous diaphragmatic flutter, convulsions, coma, and death. Urination and defecation are usually absent, intestinal motility is reduced, and swallowing is hindered.[25,26] Untreated animals usually die within 24 to 48 hours of onset.[27]

The treatment of choice is intravenous 20% calcium gluconate solution. A dose of 250 to 500 ml diluted 1:4 with saline or dextrose is usually effective for a 500-kg mare. A second dose 15 to 30 minutes later may be needed if signs do not abate.[26,27] Urination or defecation, or both, indicates a positive response.[25] Full recovery may take hours or in some cases days.[26,27] Calcium and magnesium solutions used to treat milk fever in cattle can be used with caution. Hypermagnesemia may already be present. Hypomagnesemic mares may benefit from administration of preparations containing magnesium designed for use in cattle.

The clinical signs may be mistaken for those of tetanus during the early stages, although the third eyelid does not prolapse as in tetanus and mares are not hypersensitive to loud noises.[25] Other causes of hypocalcemia should be ruled out, including blister beetle toxicity, pancreatic disease, renal disease, and exertional rhabdomyolysis.[26]

MASTITIS

Mastitis is rare in mares and occurs as isolated cases rather than as herd problems. It can occur at any stage of lactation but is most often observed after weaning[28] and can occur in mares that have not been pregnant for several years.[29] Clinical signs include a hot, swollen, painful udder; depression; anorexia; fever; ventral edema; rear leg stiffness; and lameness.[1,28,30] The milk is often thick and difficult to express from the udder because of pain. The most common organism that causes mastitis in mares is *Streptococcus zooepidemicus,* although other reported organisms include *Streptococcus agalactiae, Streptococcus equisimilis, Streptococcus viridans, Actinobacillus suis, Actinobacillus* spp, *Pasteurella ureae, Enterobacter aerogenes, Corynebacterium pseudotuberculosis, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Escherichia coli*.[29,30] Mycotic mastitis has also been reported in a mare as a result of a disseminated infection. The causative agent was *Coccidioides immitis*.[31] Mares may be affected by gangrenous mastitis similar to that which occurs in cows.[32] Verminous mastitis has been documented in a mare. The nematode identified was a species of *Cephalobus* that multiplied within the mammary gland resulting in mastitis.[33]

Treatment consists of systemic antibiotics, intramammary antibiotics, hot packs, frequent milking, and systemic nonsteroidal anti-inflammatory drugs. Antibiotic choice should be based on culture and sensitivity of the organism. However, trimethoprim-sulfonamide or penicillin and gentamicin are good choices for initial systemic therapy while awaiting culture results.[28] Bovine intramammary infusions can be used in the mare's udder, but the size of the teat orifices and the pain involved in many cases make administration difficult. Both orifices on each teat should be treated if intramammary infusions are used, because each orifice leads to a separate lactiferous duct system within the gland. Treatment can be unsuccessful and result in persistent infection and atrophy of the glandular system.

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CHAPTER 16

Immediate Care of the Postpartum Mare and Foal

MICHELLE M. LEBLANC

EXAMINATION OF THE NORMAL POSTPARTUM MARE

The initial examination of the postpartum mare should be simple, as intervention beyond absolute necessity may disrupt the adaptation processes that are under way during this time. The examination should consist of evaluation of the mare's behavior including attitude and interaction with her foal and her general condition including character of pulse and respiration, color of mucous membranes, degree of alertness, and responsive reaction to stimuli. The udder also needs to be carefully examined for consistency of mammary secretions and patency of the teats. Further evaluation of the systemic condition, such as rectal and vaginal examination, blood counts, and clinical chemistry tests, are not indicated unless a specific problem is suspected based on the general examination.

The placenta should be thoroughly examined and weighed once it is passed. A normal equine placenta weighs approximately 14% of the mare's body weight or between 10 and 18 lb. A placenta weighing greater than 18 lb is edematous and indicates that the foal may not have received adequate gas exchange in utero. Foals from excessively heavy placentas need to be considered at high risk for neonatal problems. The chorioallantoic and allantoamnionic surfaces and the umbilical cord need to be examined. Irregularities in color, thickness, length of villi, and the presence of any secretions should be noted. If placental abnormalities are found or the foal is born before 325 days of gestation, a blood sample from the foal should be obtained and a complete blood count performed. Foals that experience in utero stress may have either a low white blood cell count (<5000 cells/μl) and low fibrinogen level (<200 mg/dl) if the stress was of short duration leading to premature delivery, or if the stress was

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prolonged, a high white blood cell count (>8000 cells/μl) and a high fibrinogen level (>400 mg/dl).[1] Premature foals or foals that experience in utero stress have a greater chance of survival with appropriate nursing care.

CARE OF THE NORMAL POSTPARTUM MARE

Mares should foal in a clean, dry, draft-free area that has protection from excessive sun and wind. If the climate permits, a small, clean grass paddock is best; otherwise, a well-bedded dry stall that is at least 12 ft by 12 ft will do. Mares housed in paddocks can be grouped with either one or two mares or be left by themselves. The number of mares in the paddock should be minimal to decrease competition among the mares for food and space and to allow the mare to bond with her foal.

During the postpartum period mares need exercise to promote uterine involution and to stimulate appetite and gastrointestinal function. Leaving a mare in a stall for prolonged periods is detrimental, as the mare may accumulate intrauterine fluid leading to metritis or septicemia. If the mare must remain in the stall because the foal is ill, the mare's uterus should be evaluated daily for fluid accumulation. If fluid accumulates, lavaging her uterus with large volumes of warm saline until the efflux is clear followed by administration of 10 to 20 units of oxytocin has been helpful in preventing metritis.

For the first few days after foaling, feeding should be light to moderate, and laxative feeds such as bran mashes are appropriate to reduce the incidence of constipation.[2] Routine care of the mare post partum should include essential preventive medicine procedures. In the ideal situation, mares will have received routine vaccinations for the common infectious diseases during the last month of gestation. This allows maximum protection for the foal by way of colostrum. When vaccination history is vague or absent, the mare should be simultaneously vaccinated with tetanus antitoxin and toxoid, at different sites.

Most broodmares on well-managed farms are on a parasite control program whereby antiparasiticals are given every 45 to 60 days. If the mare is not on a bimonthly program and has not been dewormed during the last 2 months of gestation, she should be dewormed within a few days of foaling. Broad-spectrum antiparasitical compounds such as ivermectin are best. Then, an intensive parasite control program, preferably deworming every 45 days, should be implemented.

Mares with a history of a Caslick's operation as an essential part of infertility management should be resutured as soon as practical. If performed within 15 minutes of parturition, local anesthesia is not required. If the mare tears the dorsal commissure of her vulva and it is not sutured immediately, it is best to keep the area clean until it is sutured in 3 to 4 days. If it is sutured when inflammation is maximal, 24 to 48 hours after parturition, it will likely dehisce.[3]

COLOSTRUM MANAGEMENT

As the foal depends on absorption of adequate quantities of colostral immunoglobulin for protection against disease during the first month of life, the quality and quantity of colostrum needs to be assessed. Colostrum with a high immunoglobulin concentration is thick and sticky with either a yellow- or gray-tinged appearance. Immunoglobulin content can be estimated by measuring the colostral specific gravity. However the equine colostrometer (Lane Manufacturing, Loveland, CO) developed for measuring specific gravity is difficult to obtain commercially. A colostral specific gravity of 1.06 or greater correlates with a colostral IgG content of greater than 3000 mg of IgG/dl (30 G/L). Foals that suckle colostrum with a specific gravity over 1.06 rarely exhibit failure of passive transfer and have serum IgG concentrations above 400 mg/dl at 24 hours of age.[4] Colostral quality can also be estimated with a sugar (Bellingham & Stanley, Inc., 5815 Live Oak Parkway, Suite 2C, Norcross, Atlanta, GA 30093) or an alcohol refractometer.[5] The alcohol refractometer is used to measure the percentage of alcohol in wine by wine makers and is readily available. Colostrum with a level of 6000 mg of IgG/dl (60 g/L) read 16% with the alcohol and 23% with the sugar refractometer.

Colostrum with a specific gravity above 1.07 or with a 16% reading from the alcohol refractometer or 23% with the sugar refractometer may be saved for a colostrum bank. Two hundred fifty milliliters can be collected from the udder after the foal first sucks. The colostrum should be tested for isoantibodies to ensure that the foal that receives the banked colostrum does not develop neonatal isoerythrolysis. Colostrum can be stored in clean labeled containers in a refrigerator freezer (–5° C) for approximately 18 months without degradation of the IgG. Frozen colostrum can be thawed in warm water or in a microwave on the defrost cycle.

TREATMENT OF MARES WITH POSTPARTUM COMPLICATIONS

Postpartum mares that are ill will usually exhibit signs of abdominal pain or depression. Examination of these mares must include a complete physical, transrectal palpation of the reproductive tract and manual vaginal examination. Transabdominal or transrectal ultrasonography can be used to identify a ruptured bowel or uterus, the presence of free blood or feces in the abdomen, or peritonitis. Ancillary clinicopathologic tests should include a complete blood count, serum electrolyte determinations, and in patients in which internal bleeding or compromised bowel function is suspected, abdominocentesis and cytologic evaluation of the fluid obtained.

Diagnosis is often difficult because the clinical signs of many postpartum problems are nonspecific. Abdominal pain is the most common clinical sign of periparturient mares experiencing difficulty. This sign frequently occurs in foaling mares undergoing normal uterine involution and expulsion of the placenta. However, because of the incidence of complications, the following differential diagnoses must be considered in mares showing signs of abdominal pain after foaling: internal hemorrhage from rupture of the uterine artery or uterus; rupture of the cecum, stomach, or right ventral colon; ischemic necro-

sis of the small colon; colonic torsion; uterine torsion; retained placenta; and rupture of the urinary bladder or diaphragm.

Depression may follow a course of abdominal pain and may be the only clinical finding, especially if the mare has ruptured a viscus or has septic metritis.[3] These conditions can usually be differentiated by physical examination, complete blood count, and abdominocentesis.

Postpartum Hemorrhage

Hemorrhage from a uterine artery is common in older mares and is a cause of death in a significant number of aged broodmares.[2] Multiparous mares over 10 years of age are primarily affected; however, postpartum hemorrhage may occur in young mares as well. Hemorrhage from the artery is not always fatal. It may slowly dissect into the broad ligament or between the myometrium and the serosa of the uterus, forming a hematoma. The resulting clot stops the arterial bleeding and the mare may not exsanguinate. If the broad ligament ruptures or the serosal surface of the uterus tears during formation of the hematoma, the mare quickly bleeds to death.

Mares that bleed into the broad ligament display signs of colic. As tension increases on the broad ligament and the uterine serosa stretches, the mare sweats, the pulse rate increases, and the mucous membranes become pale. Signs of colic may go unobserved, if parturition has been normal and it is assumed that the mare is exhibiting pain from normal postfoaling uterine contractions. Many mares with postpartum hemorrhage are not discovered until they are weak or dead.

Hemorrhage into the broad ligament can be diagnosed by transrectal palpation of the uterus and ipsilateral broad ligament and ovary or by transabdominal ultrasonography of the caudal abdomen. Palpation causes extreme discomfort, and the degree of enlargement of the uterus indicates the extent of hemorrhage.

Mares that have uterine artery tears do not always have a low packed cell volume (PCV) early in the course of disease because the spleen may contract and thereby increase the number of red blood cells in the intravascular space. The PCV of peritoneal fluid may range from 15% to 50%, depending on the amount of blood that has leaked into the peritoneal cavity. Mares with a ruptured uterus may also exhibit blood in the peritoneal tap. Over time, these mares exhibit an increase in number of white blood cells and possibly bacteria in their peritoneal fluid.

The most successful treatment consists of confining the mare to a dark, quiet stall, using mild sedation if necessary. Blood transfusions, plasma volume expanders, and fluid therapy do not seem to alter the course of many cases and may even be contraindicated if the mare becomes excited by treatment procedures. Administration of naloxone (8–32 mg) or formalin (10 ml) in 500 ml of saline intravenously has gained popularity as a treatment for controlling hemorrhage in the postpartum mare, but the efficacy of these treatments is not clear. The recommended dose of naloxone is not sufficient to stop hemorrhage but at the low dose mares experience a profound relaxation.

Rupture of the Uterus

Mares that rupture the dorsal aspect of the uterus during second stage labor frequently stop active contractions during the delivery. These mares quickly become depressed, are cold to the touch, and sweat. Some will exhibit signs of colic. If the uterine tear is located elsewhere, as in the tip of the horn, the mare may not show clinical signs for 12 to 24 hours. Again, clinical signs will range from acute colic to severe depression. Unless the tear is located just anterior to the cervix or within the uterine body, it is difficult to find the lesion on manual exploration of the uterus. Abdominocentesis is most helpful in confirming the diagnosis. Mares with a uterine tear will have extracellular or intracellular bacteria, increased numbers of white blood cells and red blood cells, degenerate neutrophils, and increased protein content in the peritoneal fluid.

Immediate treatment for hemorrhagic shock or dehydration is indicated. Antibiotics and nonsteroidal anti-inflammatory drugs should be given to control sepsis. The best treatment for salvaging both the life and breeding potential of the mare is laparotomy and surgical repair of the uterine rupture. Abdominal lavage can be performed to reduce contamination. Following repair the uterus should be massaged every 24 hours to prevent formation of adhesions.[6]

Gastrointestinal Complications

Gastrointestinal complications in postpartum mares include rupture of the cecum or right ventral colon, colonic torsion, and ischemic necrosis of the small colon.

The large bowel may be traumatized during parturition when it is full of ingesta or distended with gas. Abnormal expulsive efforts are strong enough to rupture portions of the large bowel entrapped in the birth canal. The cecum is the most likely organ to be ruptured, apparently by explosive increases in intra-abdominal pressure. The large colon and possibly even the rectum may be damaged during foaling. Rents commonly occur 10 to 15 cm from the ileocecal orifice, ventrally in cecal rupture, or caudally in ventral large colon rupture.

Affected mares appear normal at the beginning of parturition. However, during the second stage of labor, instead of the normal, powerful straining using thoracic and abdominal muscles, straining movements are either weak or absent. Manual examination reveals a large fetus in normal presentation, position, and posture, but the mare appears unable to expel it. Manual delivery of the foal is accomplished without difficulty but subsequent observation shows the mare is not recovering from foaling as expected. Increased pulse and respiration rates, sweating, and shock due to severe peritonitis develop quickly.

Most mares succumb about 4 to 6 hours after delivery of the foal. Abdominocentesis is an important diagnostic tool in such cases; the mare should be destroyed if ingesta is discovered in the peritoneal cavity. The course of those mares in which the large bowel is damaged but not ruptured is less dramatic. The ensuing necrosis results in a

slowly developing peritonitis due to movement of organisms through the damaged gut wall.

Though treatment for rupture or severe trauma to the large bowel is virtually impossible, some preventive measures before foaling are helpful. Though most mares voluntarily restrict roughage intake within a few days of parturition, some do not. This is particularly true when mares are managed in groups that compete for feed. It is a good management practice to reduce the quantity of hay fed to all mares a few days before parturition.[2]

Contusions of the small intestine or small colon or its attaching mesentery can occur during parturition. Contusions may produce mild transitory signs of colic and go unrecognized. More severe trauma may result in mesenteric rupture, leading to possible intestinal incarceration or occlusion of the vascular arcade, which results in segmental ischemic necrosis of the bowel ("bruised small colon"). Signs of abdominal pain are seen at any time, from foaling to 24 hours after parturition. Rectal palpation of abdominal viscera reveals inconsistent and varied findings, from absence of palpable abnormalities to an impacted small colon. Results of peritoneal fluid analysis are consistently abnormal.

Exploratory celiotomy is recommended if mesocolic rupture is suspected. However, resection of the devitalized bowel may be complicated by difficulties in surgical exposure, depending on the anatomic location of the injured bowel. Survival rates following ischemic necrosis of the small colon are poor.

IMMEDIATE CARE OF THE NEWBORN FOAL

The role of the veterinarian in the immediate postnatal period will vary with training and experience of the foaling attendants. As the veterinarian is seldom present for a normal foaling, he or she should review with the foaling attendant normal foal behavior and emergency procedures. Guidelines indicating when veterinary assistance is needed should be discussed with the foaling assistant. If parturition proceeds normally, the first veterinary examination is conducted between 8 and 24 hours after birth. Normal foal parameters are presented in Table 16-1.

A foal that is not breathing at birth needs immediate assistance. The foaling attendant can attempt to resuscitate the foal by clearing the nostrils and mouth, by placing blunt objects into the nostrils to stimulate breathing, and by holding the head upright so that fluid may drain through the nostrils. Mouth-to-nose resuscitation may "buy time." The veterinarian should be contacted immediately. Large farms frequently have a source of humidified oxygen that may be delivered to foals. Farm personnel must be trained in its use.

Immediately after birth and again in 4 to 6 hours, the navel of the foal needs to disinfected to reduce the number of microorganisms that colonize the umbilical stump. For years, an iodine based disinfectant, preferably a 3.5% solution, has been advocated. Stronger solutions such as 7% tincture of iodine cause tissue destruction and should be avoided. Work from California, however, indicates that a 0.5% chlorhexidine diacetate solution is more effective in reducing bacterial numbers than 2% povidone-iodine and does not cause tissue destruction.[7]

Table **16-1**

Parameters of Normal Foals Immediately after Birth

Parameter	Time Frame
Time to suck	Within 2–20 minutes; stimulated by placing finger in mouth
Sternal recumbency	1–2 minutes
Time to stand	1–2 hours; longer than 2 hours is abnormal
Time to nursing	2 hours; longer than 3–4 hours is abnormal
Temperature	99–101.5° F
Heart rate	1–5 minutes post foaling >60 bpm; 6–60 min: 80–130 bpm
Respiration rate	First 30 minutes post foaling: 60–80 breaths/min; 1–12 hours: 30–40 breaths/min
Blood Glucose	>80 mg/dl

Adapted from Madigan JE, DVM, Live Oak Publ, Woodland, CA 95776.

Warm water enemas or soap-based enemas are commonly administered to foals to facilitate passage of the meconium. If used, the enema tube should be lubricated before its placement in the rectum. Small amounts of enema fluid, 60 to 120 ml, should be administered slowly, and repeated until the meconium is passed. If resistance is met during delivery of the enema, the procedure should be stopped until veterinary assistance is available.[8] If the dam has not been vaccinated against tetanus during the last 30 days of gestation, her foal should receive tetanus antitoxin at birth. Tetanus toxoid should be given at 6 weeks of age and repeated at 12 weeks. Antibiotics are not indicated if the foal is normal at birth.

The foal's first veterinary examination is usually between birth and 24 hours. At this time the veterinarian should observe the foal from a distance to determine its behavior, ability to rise, coordination and strength, ability and willingness to nurse, and attitude and response to external stimuli. A brief but complete physical examination should be performed.[9] A serum sample for measuring IgG concentration and, if a problem is detected in either the foal or the placenta, a blood sample for a complete blood count needs to be drawn. Foals with serum IgG concentrations above 800 mg/dl are considered to have adequate transfer of maternal immunity. Foals having serum IgG concentrations below 400 mg/dl are considered to have failure of passive transfer. Serum for measuring IgG can be obtained as early as 8 hours after birth. By measuring serum IgG concentrations in foals at 8 to 12 hours of age the veterinarian has time to supplement orally foals with low IgG concentrations prior to gut closure. Foals younger than 18 hours of age with IgG concentrations between 200 and 400 mg/dl and foals whose dams have colostral specific gravities less than 1.06 (alcohol refractometer reading <16%; sugar refractometer reading <23%) should be supplemented with at least

250 ml of colostrum that has a specific gravity greater than 1.06. Orphan foals, foals whose dams prematurely lactate, and foals with IgG concentrations below 200 mg/dl may need up to 1 L of colostrum. On a weight basis, foals require approximately 1 g of colostral IgG/kg of body weight to attain an IgG concentration of 800 mg/dl serum.[4]

Therapies for foals older than 24 hours of age with failure of passive transfer (FPT) include intravenous plasma, purified IgG products, and antibiotics. It must be remembered that the specificity of IgG administered may be more important in preventing infection than the total concentration of IgG attained in the foal's serum. Therefore, some commercial products may not contain antibodies to the potential pathogens in the foal's environment. The ideal plasma donor is an adult horse with serum IgG concentrations greater than 1500 mg/dl that has been blood typed and found free of isoantibodies to the equine major blood types (universal donor).

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CHAPTER 17

Infectious Diseases of the Puerperal Period

CLAIRE CARD and CHERYL LOPATE

The puerperal period is the time between the birth of the foal and expulsion of the placenta. This period is normally brief and is often complete within a matter of hours. Involution of the uterus is extremely rapid and by 12 hours post partum the previously gravid horn of the uterus is 1.5 times the size of its nonpregnant state.[1,2] Infectious diseases may dramatically affect the reproductive system and prolong this period. Major disease entities include endometritis and metritis, endotoxemia, septicemia, and maternal or neonatal illness related to peripartum infection.

ENDOMETRITIS AND METRITIS

Predisposing Factors and History

Endometritis and metritis are inflammatory conditions of the uterus. Endometritis is more superficial and involves only the uterine lining, and metritis is deeper inflammation and extends to the myometrium. Both are significant diseases in mares because of frequent postpartum contamination of the uterus, the general susceptibility of the uterus to infection, the potential life-threatening compli-

250 ml of colostrum that has a specific gravity greater than 1.06. Orphan foals, foals whose dams prematurely lactate, and foals with IgG concentrations below 200 mg/dl may need up to 1 L of colostrum. On a weight basis, foals require approximately 1 g of colostral IgG/kg of body weight to attain an IgG concentration of 800 mg/dl serum.[4]

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cations of uterine infections, and the negative implications for future fertility.[1–3] Poor perineal conformation may predispose to ascending placentitis, or postpartum uterine contamination.[2,4] Susceptible mares may be predisposed to puerperal complications because of poor anatomic barriers, subsequent increased contamination with bacteria and debris, and impaired uterine contractility. A preexisting maternal infection resulting in uterine or placental disease may manifest as puerperal endometritis-metritis. Uterine inertia resulting from dystocia, uterine torsion, hydrops, abnormalities of the allantoic or amniotic fluids,[1,5–7] or endocrine imbalance may predispose a mare to puerperal infection.[8] Dystocia resulting in maternal trauma or requiring forced extraction or fetotomy may lead to contamination of the uterus with bacteria. Trauma to the uterus, cervix, vagina, or vulva during parturition may create favorable conditions for microbial overgrowth.[38] Unsanitary foaling practices, poor environmental conditions, and retention of a dead fetus, particularly after dystocia or attempted abortion of twins, may predispose to disease. Retained fetal membranes may occur as a primary condition or as a sequel to other abnormalities of parturition.

Diagnosis

The most common clinical signs associated with maternal endometritis-metritis during the puerperal period are an abnormal vulvovaginal discharge and fever. In severe cases, mares are toxemic and endotoxemic and they may exhibit any or all of the following clinical signs: inappetence, depression, fever, tachycardia, tachypnea, injected mucous membranes, ileus, vulvovaginal discharge, and an increase in the height and force of digital pulses. A physical examination should include an evaluation of the horse for signs of laminitis. Abdominocentesis may be indicated in cases in which signs of colic or abdominal pain, peritonitis, severe perimetritis, or uterine rupture and laceration are suspected. A complete blood count may reveal hemoconcentration, elevated white blood cell numbers, and an increase in fibrinogen. The differential white blood cell count often shows neutrophilia or neutropenia with a left shift and toxic changes. Serum chemistry may reveal an increase in muscle enzymes due to recumbency and prerenal azotemia due to dehydration or renal injury due to circulating endotoxins. Urinalysis, especially if done on a free catch specimen, will routinely reveal blood and increased protein due to contamination by the vaginal discharge. An examination of the reproductive tract should be complete and should include visual and digital examination of the perineum, vagina, cervix, uterus, broad ligaments, and ovaries using a combination of vaginoscopy, transrectal palpation, and ultrasonography. The perineum should be examined for bruises and lacerations. A vaginal examination may disclose any of the following: vaginal or cervical trauma including bruises, hematomas, and lacerations (particularly on the dorsal aspect); fluid pooled in the cranial vagina; vaginal hyperemia; wide dilatation of the cervix, which exposes the endometrium; the presence of retained fetal membranes; and a voluminous, watery, and fetid discharge that originates from the uterus. A subinvoluted uterus, which is overly large and soft and extends beyond reach, without palpable rugae, may be identified by transrectal palpation. Ultrasonography of the uterus allows the examiner the opportunity to evaluate the uterus and broad ligaments for hematoma formation; to evaluate the tips of the horns for rupture, inversion, or membrane retention; to determine the character and amount of free intrauterine fluid; and to evaluate the uterus for evidence of retained fetal membranes. Transrectal ultrasonography may be used to confirm the presence of partially or fully retained fetal membranes by observing a dense echogenic core in the uterus, which indicates debris or retained fetal membranes, or free floating pieces or edges of membranes. Hysteroscopy, if required to identify retained fetal membranes, may be used to directly visualize the uterine wall and is best performed after distending the uterus with fluid because it is difficult to achieve full insufflation with air or carbon dioxide in the postpartum uterus.

Examination of the fetal membranes may provide clues as to the cause of the disorder. If available, the entire placenta should be examined; however, membranes that protrude from the vagina should not be overlooked. Both surfaces of the membranes should be examined. Delivered fetal membranes should be examined for completeness and the site of rupture determined. If torn, the completeness of delivery may be assessed by reconstructing the placenta using the large vessels on the allantoic surface as guides. These fetal vessels branch from the umbilicus and encircle both horns of the placenta. A large avillous area on the chorionic surface may indicate placental separation and possibly the presence of a twin. The presence of a large demarcated region, typically green to brown in color, extending from the cervical star inward, is typical of an ascending cervical infection, while the presence of a diffuse or focal thick exudate on the chorionic surface of the uterine body or horns and clumping of the microvilli may suggest a hematogenous infection. Adenomatous hyperplasia, which grossly appears as raised round or oval firm nodules on the allantoic surface, has been associated with bacterial and fungal infection in mares.[9] Smears from an endometrial swab, exudate, or imprints from the tissue can be dried and stained.[10,11] Microscopic examination may yield clues to the nature of the disease process. A Gram stain of the exudate can be performed to determine the type of organism involved. A potassium hydroxide digestion, or periodic acid–Schiff stain with a nigrosin counterstain may be used to identify fungal elements, and a Wright-Giemsa stain is used to identify bacteria, yeast, and inflammatory cells such as macrophages and neutrophils.[11,12] Samples of the discharge should be submitted for culture and sensitivity. Fresh samples are typically more representative of the problem, as secondary opportunistic organisms frequently invade as part of the process of autolysis, may overgrow the primary pathogen, or result in a mixed culture that is difficult to interpret. Fetoplacental infection accounts for the largest proportion of fetal and neonatal losses.[13,14]

Etiology

Bacterial organisms that cause endometritis-metritis during the puerperal period include *Streptococcus equi zooepidemicus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Crossiella equi (nocardioform actinomycete), Leptospira* spp., *Brucella abortus, Salmonella abortus equi,* and *Listeria monocytogenes*. Most authors report *Streptococcus* to be the most common isolate. Gram-negative organisms are commonly isolated from cases of toxic endometritis-metritis. Mixed infections are common and anaerobic bacteria may be involved in some infections. Anaerobes require special sample culture devices for growth. The most common fungal isolate is *Aspergillus*, followed by mucoraceous fungi.[11–14] A positive postpartum uterine culture does not always indicate a clinically significant problem because potentially pathogenic microorganisms are commonly recovered from apparently healthy mares owing to normal contamination of the uterus during stage II and III of labor.[8] Culture results need to be interpreted in light of the clinical findings.

Pathogenesis

The pathogenesis of postpartum uterine infections involves inoculation with organisms through the cervix, by hematogenous spread, or by iatrogenic introduction. Fungal organisms commonly ascend through the cervix. An inflammatory process ensues and results in exudation. Under favorable growing conditions provided by dead or devitalized tissues, or in mares with impaired uterine contractility, the organisms replicate quickly and cause further inflammation with subsequent release of toxins. These toxins, if absorbed into the maternal circulation, create specific responses such as fever. Release of organisms into the circulation of the dam may occur and produce septicemia. Complications of endotoxemia and septicemia include systemic illness that may lead to death of the dam or neonate, lactation failure, impaired fertility, and laminitis.

Treatment

Treatment of endometritis and metritis in mild cases includes systemic antimicrobial therapy and multiple injections of oxytocin to encourage uterine involution. In more advanced cases, the mare's condition should first be stabilized with fluid therapy, nonsteroidal anti-inflammatory drugs (NSAIDs), and systemic antibiotic therapy. The perineum should be thoroughly cleansed and a large catheter or tube introduced through the cervix into the uterus or through the interior of the retained fetal membranes. The uterus is lavaged with saline or dilute tamed iodine solution (<1%) to remove intrauterine fluid and debris until the effluent is clear. Uterine lavage is followed by administration of oxytocin (10–40 units) to effect. Multiple small doses of oxytocin (10–20 units) may be used between lavages to help remove debris and encourage uterine involution. Uterine lavage may be repeated several times a day as long as signs of uterine disease are present. Transrectal palpation and serial complete blood counts may be used to determine the progress of the uterine therapy. Nonsteroidal anti-inflammatory drugs are indicated in cases in which a risk of laminitis is present, when signs of laminitis are present (sawhorse stance, reluctance to move, high resting heart rate that increases with locomotion, bounding digital pulses, hot hooves, extreme solar sensitivity to a hoof tester, and reluctance to lift a leg), or in cases in which endotoxemia or septicemia is present. Currently, phenylbutazone, ketoprofen, vedaprofen, or flunixin meglumine are used to treat signs of laminitis or endotoxemia-septicemia. Vasodilators such as acepromazine may be used to encourage circulation to the hooves. Bilateral palmar digital nerve blocks may be required to encourage some horses to move or to allow their hooves to be trimmed. Special hoof care for laminitic horses is advised and may involve trimming, using shoes or pads to reduce weight borne by the sole and the dorsal hoof wall, and pads to cushion the solar surface and lend frog support. Commercially available pads, shoes, and devices (Thera-Flex pads,* Lilly pads,† Farley boot‡) that can be taped on, glued on, or held in place with straps, or a horseshoe may be recommended. Radiography may be used to obtain additional prognostic information concerning laminitis. Any ventral rotation of the coffin bone relative to the dorsal hoof wall is considered undesirable, and rotation of greater than 5.5 degrees is associated with a grave prognosis. Free fluid and gas may be seen on radiographs to intervene between the coffin bone and the hoof wall in acute cases with deep abscesses in the sole.[15] The clinical signs observed in a particular animal may not relate well to radiographic signs; thus, each case should be treated on an individual basis. Dorsal hoof wall resections and heart bar shoes have been used with mixed success. Tenotomy of the deep flexor tendon has recently been recommended as a treatment for severe, intractable laminitis. Recovery from a severe case of acute laminitis that involves rotation of the coffin bone is prolonged, and athletic function may not be regained. The slow-growing nature of the hoof results in a convalescent period that may exceed 1 year. Regular hoof care is required to prevent pathologic changes seen in hooves of chronically laminitic cases. Lactation failure may be treated by the administration of domperidone (1.1 mg/kg PO once or twice a day). An increase in milk production is expected within 24 hours.

INFECTIOUS DISEASES CAUSING MATERNAL OR NEONATAL ILLNESS DURING THE PUERPERAL PERIOD

History

Pertinent aspects of history in cases of infectious disease might include illness or weight loss in the patient or in a cohort of horses on the farm, a history of new introductions, fever during pregnancy, contact with wildlife or

*Thera-Flex Pads, Thera-Flex Inc., Lawrenceburg, KY.
†Lilly Pads, Therapeutic Equine Products, Indianapolis, IN.
‡Farley Boot, Equine Orthotics Inc., Indian Habour Beach, FL.

rodents, previous episodes of vaginal discharge, premature lactation, results of other necropsy examinations, vaccination practices, exposure to pasture, presence of vectors of disease (such as Eastern tent caterpillars), and nutritional management.

Diagnosis

A complete physical and reproductive examination should be performed. Maternal samples collected when an infectious disease is suspected may include blood for a complete blood count, serology, and serum chemistry; colostrum; urine for culture or darkfield examination; exudate; feces, and examination of the fetal membranes.

Etiology of Diseases Causing Maternal Illness During the Puerperal Period

Diseases that have been reported to cause maternal illness during the peripartum period include brucellosis caused by *Brucella abortus*. Brucellosis has been reported to cause abortion, infertility, systemic illness, lameness (osteoarthritis), and suppurative bursitis in horses pastured with cattle infected with *Brucella abortus*.[16] In North America and elsewhere, infection with *Leptospira* spp. has been linked to herd problems, including poor reproductive performance and multiple cases of periodic ophthalmia.[17–20] Leptospiruria has been reported in mares that have aborted or seroconverted, and has caused outbreaks of abortion after environmental flooding.[19,20] *Leptospira* spp. may be present along with other infectious agents causing abortion.[21] *Salmonella abortus equi* has been reported to cause diarrhea and abortion in mares; however, no outbreaks of abortion have been reported in North America for a number of years.[22,23] Other strains of *Salmonella* have been associated with maternal abortion and diarrhea, particularly after stress such as hospitalization for surgery. Listlessness, weight loss, cough, and pyrexia have been described in some cases of *Mycobacterium avium* infection.[24] Rare systemic mycoses have usually been associated with maternal weight loss and include *Coccidioides* and *Histoplasma capsulatum*.[25,26] *Histoplasma* has been reported to cause abortion and granulomatous placentitis.[26] *Coccidioides immitis* was detected in two mares that aborted.[27] Equine cryptococcal placentitis and neonatal foal pneumonia have been reported.[28,29] *Candida* may infect the endometrium and cause disease.[30] Occasional transient maternal illness (fever and depression ± respiratory disease for less than 48 hours) may occur with equine herpesvirus (EHV-1) abortion. Clinical signs lasting longer than 2 days are unlikely to be caused by EHV-1.

Pathogenesis of Fetal and Neonatal Infection

Infection of the fetus occurs hematogenously or through compromise of the placenta. Perinatal infections result in stillborn or weak foals, foals with septicemia or endotoxemia, and foals with multiorgan failure. Perinatal infections may occur through the birth process *(Salmonella ohio),*[31] and through contact with maternal uterine discharge such as *Klebsiella pneumoniae* capsule type 1 and hemolytic *Escherichia coli* serotype 0101.[32,33] These latter two organisms may have an epizootic manner of transmission.

Diagnosis of Diseased Neonates

Examination of neonates should include assessment of rectal temperature, heart rate, respiratory rate, body condition, maturity (length and nature of the hair coat, stature of the ears, degree of calcification of the carpal and tarsal bones) and an evaluation of reflexes, including the righting reflex and sucking reflex. A database on a sick foal might include the following as indicated by clinical signs and economic constraints: blood for serology, complete blood count, fibrinogen, serum chemistry, blood microbiologic culture, transtracheal wash with cytologic and microbiologic culture, blood gas, urinalysis and culture, nasal and rectal swabs for virus isolation, thoracic radiographs, and thoracic and abdominal ultrasonography. Immunoglobulin levels should be measured in neonates more than 12 hours old. Foals whose dams are diseased may be born weak and thin or may become debilitated from maternal lactation failure.

Etiology of Diseases Causing Stillbirth or Neonatal Loss

It has been reported that certain diseases cause neonatal death and stillbirth. The primary list of differential diagnoses for stillbirth includes birth-related problems not necessarily caused by infectious diseases and consists of premature placental separation, suffocation due to failure of the amnion to rupture, and prematurity.[34] Infectious and noninfectious disease processes may be interrelated; hence, each individual foal should be examined by necropsy when practical. Mares may also deliver live but severely diseased neonates. A history of birth-related problems or maternal illness should be noted. A diseased neonate may come from a diseased uterine environment or dam; hence a complete examination of the mare should also be performed. Commonly encountered perinatal conditions include pneumonia, gastroenteritis, colitis, omphalophlebitis, polyarthritis, meningitis, and endocarditis due to bacteria that invade fetuses and neonates.

Viral Diseases

Viral diseases reported to cause weak or stillborn foals include equine herpesvirus type 1 (EHV-1; rhinopneumonitis).[35] Exposure to the virus may occur weeks to months prior to abortion, making maternal serologic results difficult to interpret. Foals born infected with EHV-1 often suffer terminal multiorgan failure. These foals are characteristically leukopenic and neutropenic, with total white blood cell counts below 3×10^9/L, and some are icteric at birth. They may or may not have serum titers to EHV-1. Immunohistochemical staining, polymerase chain reaction, or viral culture of lymphoid or lung tissue will establish the diagnosis. Equine arteritis virus (EVA) has been isolated from aborted fetuses and neonates.

Gross necropsy findings include edema and rib impressions on the parietal surface of the foal's lungs.[36,37] Outbreaks of EVA have been reported and include stillbirth, sudden death, and respiratory disease due to interstitial pneumonia.[38,39] Aerosol or direct contact with mares acutely infected or stallions acutely or latently carrying the virus, are believed to be a factor. In addition, fomites and bedding have been suggested to be involved in lateral transmission between mature stallions, and fomites and contaminated bedding may be a source of virus for neonates.[40]

Bacterial Diseases

Leptospirosis has become an increasingly recognized cause of neonatal loss. During a 4-year period from 1987 to 1990, the prevalence of abortion or stillbirth associated with leptospira in Kentucky ranged from 0.6% to 5.9%. Serologic studies on fetuses and mares demonstrated that 85% of the infections were caused by serotype *pomona,* with serotypes *grippotyphosa* and *Bratislava* constituting the remainder. Direct fluorescent antibody staining of fetal or placental tissues showed a positive result in 81% of the cases.[11–13,21] Warthin-Starry silver stain showed spirochetes in allantochorion or fetal kidney in all cases. Gross fetal changes ranged from an absence of lesions to an enlarged jaundiced liver, perirenal edema, radiating white streaks in the kidney, and placentitis. Histologic lesions included acute, subacute, or chronic placentitis, and subacute fetal hepatitis and suppurative fetal nephritis. Infected foals may be stillborn or born jaundiced, presumably after in utero leptospiral infection.[18]

There are also reports of isolation of *Klebsiella pneumoniae* (most commonly capsule type 1) from mares with endometritis whose foals developed diarrhea and fatal hemorrhagic enteritis caused by that organism.[32] A hemolytic strain of *E. coli* serotype 0101 was associated with metritis and foal diarrhea.[33] Infection with *Salmonella abortus equi* has been reported to occur in outbreaks and to result in septicemia and polyarthritis in newborn foals. *Salmonella ohio* infections have been reported in outbreaks with diarrhea, septicemia, and septic arthritis in newborn foals.[31] A few cases of abortion, neonatal septicemia, and meningitis caused by *Listeria monocytogenes* and cases of abortion and neonatal diarrhea caused by *Campylobacter* spp. have been reported.[42] Other rare but recently recognized diseases include borreliosis *(Borrelia burgdorferi)* in a stillborn neonate and two live neonatal herdmates;[43] however, exposure to the agent of Lyme disease does not always result in perinatal loss.[43,44] Potomac horse fever *(Ehrlichia risticii)* has been associated with abortion.[45,46] *Mycobacterium avium* has also been shown to be a cause of abortion in horses.[47]

Unknown Etiology

Mare reproductive loss syndrome (MRLS) was first reported in 2001 in Kentucky and the surrounding areas. Similar abortions had been reported in the early 1980s, but not to the extent noted in 2001 and to a lesser extent in 2002.[48,49] It is believed that unusual weather patterns (drought followed by higher than normal temperatures, followed by heavy frost) led to emergence of a higher than normal population of Eastern tent caterpillars (ETC; *Malacosome americanum*) in the region. Studies show that mares spending extensive time on pasture are at greater risk than those housed in barns. Affected farms have cases of early fetal loss, late fetal loss, pericarditis, and panophthalmitis. Mares with late term abortions have been reported to have premature placental separation, placental thickening or edema, and explosive deliveries. Lesions in the fetuses or foals include pneumonia and funisitis. Non-β-hemolytic streptococci and *Actinobacillus* organisms are routinely cultured from the fetuses or foals, but may be opportunistic organisms rather than causative factors in the abortion. Some MRLS foals are born weak with neurologic deficits and signs of neonatal maladjustment syndrome. The pathogenesis of this disease remains to be completely elucidated. The current hypothesis is that the barbed setae present on the cuticles of the ETCs are involved in the pathogenesis. The mare ingests the barbed setae fragments that are covered with or contain bacteria. The barbed setae fragments then penetrate the intestinal tissues including venules and other blood vessels, which then are proposed to embolize and to release septic material that distributes hematogenously throughout the mare. The septic materials and setae emboli spread by the hematogenous route to the placenta and fetus, and other immunologically susceptible sites such as the pericardial fluid and eye.[50] The difficulties with this hypothesis include that the venous drainage of the intestines is through the liver, and the liver has not been reported to have lesions that would be expected if embolic setae fragments were present, and bacteria have not been cultured from the blood of mares experimentally infected with the bacteria, and mares are not reported to have signs compatible with bacteremia. In 16% of the cases, no bacteria are recovered from the fetus, suggesting that bacteria may not be necessary for fetal loss.[50]

Fungal and Yeast Infections

Histoplasma has been reported to cause perinatal granulomatous pneumonia in foals.[26] A yeast organism, *Cryptococcus neoformans,* has been isolated from a fetus following abortion and from the endometrium a month later.[28] Protozoa *(Toxoplasma gondii)* have been identified by polymerase chain reaction hybridization in the placenta of a mare that delivered a healthy foal but grazed on pasture where sheep aborted because of that organism. Interestingly, the amnion had abnormal areas of vascularization and 5- to 9-mm papillae in the area of the umbilicus, similar to lesions reported in sheep.[51] *Neosporum caninum* can cause neonatal death in horses.[52]

Treatment of Diseased Neonates

In milder cases, treatment of diseased neonates includes supportive care such as administration of intravenous or oral fluids, colostrum, plasma transfusions, antibiotics, anti-inflammatory drugs, nutritional supplements, and H_2 receptor blockers. There are some reports that treatment with acyclovir (16 mg/kg PO three times a day) may

Box **17-1**

Microorganisms with Zoonotic Potential That Can Be Spread by Contact with Body Fluids, Placentas, Diseased Neonates, and Abortuses

Leptospira spp.
Borrelia burgdorferi
Listeria monocytogenes
Brucella abortus
Salmonella spp.
Campylobacter fetus
Mycobacterium avium
Coccidioides immitis
Histoplasma capsulatum
Toxoplasma gondii
Neospora caninum

help some of the EVH-1 infected foals survive.[36] Animals with advanced disease should be referred to a neonatal intensive care unit or euthanized. Necropsy is useful to obtain or confirm a diagnosis.

Zoonotic Risk

Many veterinarians view equine abortuses, fetal fluids, and placentas as a low risk for exposure to zoonotic infections. A number of infectious organisms causing puerperal disease in horses commonly infect a number of other mammalian species including humans. Box 17-1 lists pathogenic microorganisms with zoonotic potential.

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CHAPTER 18

Irregularities of the Estrous Cycle and Ovulation in Mares (Including Seasonal Transition)

KATRIN HINRICHS

Irregularities of the estrous cycle may be associated with pathology of the ovary (granulosa cell tumor, gonadal dysgenesis) or uterus (shortened luteal activity due to endometritis) or, rarely, may be due to apparent functional abnormalities of the ovarian/hypophysial axis (anovulation with hematoma formation). However, wide variations in cycle length and ovarian characteristics are associated with normal fertility. Abnormalities of endocrinology, cyclicity, and ovulation are uncommon in mares with normal reproductive tracts, and thus other causes of infertility should be ruled out before implicating abnormal cyclicity as a cause of infertility.

Apparent estrous cycle irregularities that are not associated with pathology include prolonged estrus without ovulation during the seasonal transition period, "silent heat" (apparent failure to show estrous behavior), prolonged luteal activity, and anestrus or constant estrus during pregnancy. Additionally, aging of mares is associated with changes in cyclicity.

Abnormal behavior may be related to ovarian pathology, most commonly granulosa cell tumors, in mares. Because this link is known, many other behavioral changes may be attributed to the ovaries by mare owners when in fact they have no association with reproduction.

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Abnormal behavior may be related to ovarian pathology, most commonly granulosa cell tumors, in mares. Because this link is known, many other behavioral changes may be attributed to the ovaries by mare owners when in fact they have no association with reproduction.

For this reason, mares with abnormal behavior may be presented for examination of the reproductive tract, and it becomes a diagnostic challenge on the part of the theriogenologist to determine the cause of the change in behavior.

Diagnosis of the cause of estrous cycle irregularities in mares is based on history, teasing records, findings on palpation and ultrasonography per rectum, and determination of hormone concentrations, when indicated.

ASSESSMENT OF THE ESTROUS CYCLE AND OVULATION

The Estrous Cycle

A complete review of mare endocrinology is given in Chapter 7. An important factor to remember when evaluating apparent cycle abnormalities is the normal variation in length of estrus. Estrus length in normal cycling mares can vary at least from 2 to 12 days. Estrus length is generally repeatable within mares, and is longer at the beginning and end of the breeding season (the peak of the breeding season being the first day of summer, June 21 in the Northern Hemisphere). In some mares, estrus becomes so short in midseason that it may be missed if teasing is only performed sporadically. Owners may report that their mare was showing good heat in April and May but that they haven't seen her in heat in June.

Teasing is a major determinant of apparent cyclicity. Individual teasing of mares with an active stallion is the best method of heat detection. To adequately detect cyclical changes in behavior, teasing should be performed thoroughly at least three times weekly, and the mare's behavior scored by a knowledgeable individual. "Silent heat" may occur in mares that have normal ovarian function. The meaning of this term is relative, indicating only that the observer did not recognize estrous behavior. Knowledge of the estrous and diestrous reactions of each individual mare is crucial in heat detection; one mare in heat may be less demonstrative than another mare in diestrus. Some mares may show signs of estrus immediately on contact with the stallion; others—especially young or timid mares—may require teasing for 3 to 4 minutes before they respond. A mare that shows no change in behavior at all during her cycle, as assessed by an experienced observer using good heat detection methods, may present a challenge. Such a mare may need to have the reproductive tract examined regularly by transrectal palpation and ultrasonography to detect estrus (see later discussion). However, assessing the willingness of the mare to allow mounting by a stallion may reveal that she is receptive to him, even though teasing evoked no signs of estrus. Mares that do not show good signs of estrus to a stallion may begin to show estrous behavior if they are bred by natural cover, during estrus, on a few occasions.

Estrous behavior in mares does not necessarily indicate follicular activity. Estrous behavior can be seen in mares in seasonal anestrus, in mares that have been ovariectomized, and in mares with gonadal dysgenesis. This is because sexual behavior in the mare is largely regulated by progesterone; that is, estrous behavior is suppressed by progesterone. In the absence of progesterone, even the small amount of estrogen produced by the adrenals is sufficient to cause the mare to show some signs of estrus. Further stimulation with estrogen does, however, increase the intensity of estrous behavior. This is reflected in the finding that mares in true estrus (during the follicular phase of the cycle) have fewer negative reactions to mounting or intromission than do seasonally anestrous or ovariectomized mares.[1]

The length of diestrus is more repeatable among mares than is the length of estrus, at 15 ± 2 days.[1] When evaluating heat detection records, repeated diestrus intervals of normal length, interspersed with estrous intervals, are a good indication that corpora lutea are forming and being lysed normally. The formation of corpora lutea in turn suggests that normal follicle growth and ovulation are occurring.

Hormone analysis can also be helpful in defining the pattern of cyclicity in a mare; progesterone concentrations are of most value. Reflecting teasing records, a pattern of high progesterone (increasing to >4 ng/ml) for approximately 14 days followed by low progesterone (<1 ng/ml) for 3 or more days is strongly indicative of normal cyclicity.

Ovarian and Uterine Characteristics of Normal Cyclicity

In assessing the normality of follicle growth and ovulation, the large variation in follicle size at ovulation should be recognized. The size of the follicle the day before ovulation is detected is commonly 35 to 45 mm in diameter, but mares can ovulate much smaller follicles (less than 30 mm), or larger follicles, with normal fertility. Follicle size at ovulation is often repeatable for a given mare. Small follicle size at ovulation may be a cause of apparent infertility because, unless this tendency is known, the mare may not be bred at the appropriate time. When double ovulations occur, the follicles ovulate at a smaller size than for single ovulations. Follicle size at ovulation also decreases toward the middle of the breeding season (July and August). Numerous large follicles and corpora lutea are normally present on the ovaries of pregnant mares, especially between 30 and 120 days. These are sometimes mistaken for ovarian pathology.

Transrectal palpation and ultrasonography are indispensable tools in the evaluation of the estrous cycle. Familiarity with the normal appearance of the ovary and uterus on ultrasonographic examination throughout the cycle is a great help when assessing possible abnormalities.[2] Multiple follicles may be present on the ovaries at any stage of the cycle. During estrus, the dominant preovulatory follicle is usually identifiable at approximately 25 mm diameter, when it is about 5 mm larger than other follicles on the ovary. In some mares, however, multiple follicles appear to grow to a large size (>30 mm) from which one or two ovulate; this is associated with normal fertility. On ultrasonographic examination, follicles usually are round and should be echolucent (black) in appearance. In the day or so before ovulation, infrequent small echodensities may be seen within the follicle, and

the follicle may become less round, reflecting a palpable loss of tone. Immediately before ovulation, follicles may appear pointed on ultrasound examination, as they apparently protrude toward the ovulation fossa.

On the first day after ovulation, the collapsed follicle may be difficult to define ultrasonographically. The only finding may be that the follicle seen previously is gone; however, a thick echodense line or mass is usually visible within the ovary. At this time, a depression may be palpable on the ovary, and the ovary may be sensitive to palpation. The normally developing corpus luteum (CL) has two major forms on ultrasonographic examination.[2] Over the first few days after ovulation, the CL may become more and more apparent as an echodense structure within the ovary; then, after about 7 days, it may become less dense and less easily delineated. Alternatively, the ovulated follicle may fill with clotted blood over 1 to 2 days. This corpus hemorrhagicum has a typical ultrasonographic appearance of a round structure with an echodense rim and an echolucent interior, crossed with a lattice of echodense fibers. The echolucent area decreases in size over the next few days as the echodense rim thickens; after 5 or more days this structure is indistinguishable from the CL that did not have an interior clot. A normal corpus hemorrhagicum is typically no larger than was the original follicle; a larger structure with similar appearance on ultrasonography may be a hematoma (see later discussion).

Uterine changes detectable on ultrasonographic examination are also helpful in staging the cycle in mares with questionable estrous behavior. The uterus in estrus becomes edematous. This is characterized on palpation as a heaviness and lack of tone, and ultrasonographically by presence of echolucent areas of fluid within the uterine folds, giving a "wagon wheel" or "orange slice" effect to the cross-sectional uterine image. In diestrus, the uterus is homogenous in appearance on ultrasonographic examination and tends to be more tightly circular in cross section, reflecting increased tone. Changes in palpable cervical tone reflect those of the uterus but may be more influenced by individual variation between mares; cyclical ultrasonographic changes in the cervix are not obvious.

PHYSIOLOGIC ESTROUS IRREGULARITY—THE TRANSITIONAL PERIOD

The mare is a seasonal, long-day breeder, and the majority of mares enter anestrus in the winter. Ten to 25% of mares may cycle through the winter, this percentage being higher in mares having good nutrition and housing. In anestrus, gonadotropin-releasing hormone (GnRH) and gonadotropin secretion is low; follicles reach a maximum diameter of 10 to 15 mm and then regress. The period between anestrus and the first ovulation of the year is termed the transitional period. During this time, pituitary gonadotropin output is sufficient to cause follicular growth, but insufficient to stimulate normal follicle maturation and ovulation. The follicles produced at this time are indistinguishable from normal follicles on palpation and ultrasonographic examination; however, they may be structurally and hormonally abnormal. For this reason, transition follicles will not ovulate in response to one-time administration of gonadotropins or GnRH analogues.

During the transitional period, mares may be presented for examination because of constant or irregular estrus, as they respond to rising and falling estrogen from waves of nonovulatory follicles. Owners may become frustrated at this time with breeding mares over periods of weeks as the mares remain in heat. When estrous behavior does subside, it may recur within days; there is no normal diestrus interval. Unless the mare is bred immediately before the first ovulation of the year, the breedings will not result in pregnancy.

On palpation and ultrasonographic examination, the ovaries have multiple small (in early transition) to large (in late transition) follicles. Extreme cases may have many (5–10) follicles greater than 30 mm in diameter on each ovary. No corpora lutea are present on the ovaries; however, corpus hemorrhagicum–like structures may be present, as these anovulatory follicles sometimes become filled with blood. The structures do not luteinize and so do not progress over time to the normal CL appearance on ultrasonography as described previously. The lack of luteal progesterone (<1 ng/ml in peripheral blood throughout this period) is reflected in poor uterine tone. Some practitioners feel that the presence of uterine edema signals the presence of a functional preovulatory follicle; however, recent studies have not supported this relationship. As gonadotropin secretion reaches functional levels, one of the follicles on the ovary will grow and ovulate; this signals the end of transition and the mare will cycle normally through the season from this point.

Irregular estrous behavior and multiple anovulatory follicles are a normal aspect of the transition period and do not represent an abnormality. Diagnosis of seasonal transition is based on the season of the year (usually February through April in the Northern Hemisphere), lack of evidence of ovulation (no diestrous periods; no corpora lutea; poor uterine tone; low progesterone on assay), and multiple small to large follicles present on both ovaries.

MANAGEMENT OF SEASONAL TRANSITION

Because of the January 1 birthdate imposed by many breed registries, foals born in a given year compete with one another. This has resulted in a demand for foals to be born early in the year. To have a foal born in January, the mare must conceive the previous February, during the period of seasonal transition. Many management methods have been developed to shorten the transitional period or move it to an earlier time of year (December to January). Currently no method is both commercially feasible on a large scale and highly predictable; however, it is likely that in the future this may be achieved using some combination of the treatments currently under investigation.

The most widely used method for management of seasonal transition is increasing perceived day length. This

may be done by housing mares under artificial light (100 lux, or equivalent of a 200-watt bulb 12 ft from the ground in a 12 ft by 12 ft stall) starting approximately December 15. Mares should be exposed to 14.5 to 16 hours of light per day; there is no benefit to increasing the apparent day length slowly, and lengths of light greater than 20 hours may actually reduce effectiveness. Alternatively, it has been shown that a short (1- to 2-hour) burst of light timed to occur at 9.5 to 10.5 hours after dusk is equally effective as having light on constantly for 16 hours. Although this technique saves electricity and may allow large groups of horses to be treated, application is more difficult because the time of the light burst must be correlated with changing day length. Recent studies have shown that reduced lux and reduced treatment periods may be equally as effective as is the traditional regimen.[3] Mares typically ovulate and resume normal cyclicity 6 to 10 weeks after the onset of increased day length.

Use of GnRH administration to induce cyclicity in anestrous or seasonal transition mares has been studied extensively.[4] Infusion of pulses of GnRH (25 to 250 μg/hour) at hourly intervals results in ovulation in essentially all anestrous mares within 11 days. This ovulation may be fertile, but mares may not continue to cycle if not pregnant. Although native GnRH is commercially available, this application requires the attachment and use of a pump. Constant infusion of GnRH or its analogues may also be effective, but responsiveness appears to be greater when the mare has entered transition. Constant infusion of GnRH using an osmotic minipump (an implant that releases drug as it accumulates fluid osmotically), delivering 100 to 200 ng GnRH per kg body weight per hour, induced ovulation in 60 to 70% of mares within 30 days. Injection of long-acting GnRH analogues (e.g., 40 μg buserelin twice daily) induced ovulation in 50% of anestrous mares in 10 to 25 days; however, this compound is not available commercially in the United States. Use of the commercially available implant containing 2.2 mg of the GnRH analogue, deslorelin, administered every other day has been shown to induce ovulation in about 50% of transitional mares. However, recent reports that deslorelin implants suppress pituitary responsiveness to native GnRH after administration suggest that repeated use of the implant may not be advisable. Indeed, when deslorelin was used to induce cyclicity, ovarian suppression was noted in the treated mares that did not ovulate.

Injection of equine pituitary extract (once daily for 14–20 days; dosage is dependent on strength of the preparation) is effective for induction of follicular growth and ovulation in anestrous mares, but this compound is not currently commercially available.

Progesterone and its analogues (e.g., altrenogest) have been used in an attempt to induce early cyclicity with the thought of suppressing luteinizing hormone (LH) release from the pituitary and then having a "rebound" when the progesterone is withdrawn. Progestins are effective in suppressing estrous behavior in transition mares and for this reason may be used simply to reduce the client's concern about whether to breed the mare. The progestin is typically given for 10 to 15 days. Mares return to estrus approximately 3 days after the progestin is withdrawn, because the suppressive action of progesterone on estrous behavior is lost. Administration of progestin is not effective in hastening ovulation if given to mares in anestrus or early transition. In late transition, the first ovulation of the year in mares treated with progestin (e.g., 0.44 mg/kg altrenogest for 12 days) may be hastened by about 10 days.

Other methods for inducing cyclicity during seasonal transition are currently under study. Dopamine antagonists, such as sulpiride (0.5 mg/kg, IM) or domperidone (1.1 ng/kg PO) daily for 10 to 60 days have been shown to induce cyclic activity in seasonally anestrous and transition mares.[3] However, a repeatable method for use of these compounds has not yet been developed, and the response seems to depend on mare, environmental conditions, and stage of transition at the time of treatment. The variability in effect may be due to the mechanism of action of dopamine antagonists in the mare. In contrast to its action in ewes, dopamine antagonists do not appear to directly increase gonadotropin secretion in mares. Dopamine antagonists may work via increasing prolactin concentrations, which sensitize the ovary to circulating gonadotropins;[3] therefore, there must be some native gonadotropin activity for ovarian response to occur.

CYCLE IRREGULARITIES ASSOCIATED WITH AGING

Mares cycle less efficiently after about 20 years of age. There is a delayed entry into the ovulatory season, longer follicular phase (estrus), and fewer ovulations per year.[5] Mares over 25 years of age may cease cycling altogether. These mares may be presented for examination because of erratic or constant heat during the breeding season, or because multiple breedings have not resulted in pregnancy. They may also be apparently anestrous during the breeding season.

Diagnosis is based on the mare's age; these changes are not usually seen until the mare is near 20 years of age. The delay of the first ovulation of the year is associated with a prolonged transitional period in the spring, which extends into the normal breeding season. Breedings during this time will not result in pregnancy because the mare is not ovulating. Findings on palpation and ultrasonographic examination will be similar to those described earlier for the transitional period. Once the mare does ovulate, however, the corpus luteum appears to have normal function and cycles may be regular. Old mares that have ceased cycling will appear to be anestrous (have no follicular activity, no uterine tone, and low progesterone) throughout the breeding season.

Management of old mares that are still intended for breeding would include monitoring the ovaries by palpation and ultrasonographic examination over time to detect follicular growth leading to ovulation. In the case of an old mare that is anestrous, treatment with pulsatile GnRH may be effective in inducing follicular growth and ovulation. It must be remembered, however, that even when cycling normally, old mares have low fertility,

associated with decreased oocyte viability and uterine changes.

Prolonged Luteal Activity

A prolonged luteal phase, that is, greater than 17 days, is a common occurrence in mares. It is estimated that failure of normal return to estrus may occur in 4% to 18% of cycles, and in these cases the luteal phase may be prolonged to over 60 days.[1] The first work in this area was done before ultrasonography, and it was presumed that the primary corpus luteum (from ovulation during the previous estrus) was somehow maintained after the normal time of luteolysis. Work with ultrasonography has shown that a prolonged luteal phase is seen when a diestrous follicle ovulates after day 10 of diestrus. This new corpus luteum is too immature to respond to the endogenous prostaglandin release around day 14. Persistent luteal activity can also be associated with an apparently luteinized hematoma. It is not known whether idiopathic maintenance of the primary corpus luteum can also be a cause of prolonged diestrus.

Another possible cause of prolonged luteal activity is severe damage to the endometrium, as seen in overt pyometra. If the damage is severe enough that prostaglandin production is impaired, retention of the primary corpus luteum results. Inflammation of the endometrium, as seen in endometritis, may result in release of prostaglandin and thus shortening of the luteal phase (see later discussion).

Mares with prolonged luteal activity may be presented because of failure to return to estrus. The other major differential diagnoses for failure to return to estrus are pregnancy, which can happen even if no breeding is recorded; silent heat (the mare that is cycling but does not show behavioral estrus); and poor estrus detection, or short heats that are missed, especially near the middle of the breeding season. Diagnosis of prolonged luteal activity is based on finding a normal-appearing nonpregnant diestrous tract with failure to show estrus or have estrus-type examination findings for more than 17 days after ovulation. Progesterone concentrations will be high (usually >4 ng/ml) for more than 2 weeks.

Treatment of prolonged luteal activity is simple and effective: administration of prostaglandin F2α (10 mg IM). To assure a response, the prostaglandin should be given at least 5 days after the most recent ovulation. The mare should return to heat in about 3 days. It should be verified that the mare is not pregnant before treatment, even if the mare has had a previous negative pregnancy check.

Shortened Luteal Phase

A decrease in the length of diestrus (less than 13 days) may be indicative of premature luteolysis. Primary defects of the corpus luteum have not been confirmed in the mare.[1] The most common factor associated with premature luteolysis is endometritis; prostaglandin production associated with uterine inflammation or bacterial endotoxin production is the causative factor. Some interval of diestrus is seen even if an active endometritis is present, as the corpus luteum is not completely responsive to prostaglandin for about the first 5 days after ovulation. After this, the relatively low but constant production of prostaglandin is sufficient to cause lysis of the corpus luteum over a period of days, typically resulting in a 7- to 11- day diestrus. If a shortened luteal phase is detected, an endometrial culture and biopsy should be obtained to determine if endometritis is present, and, if so, which organism may be responsible. Resolution of the endometritis should result in return of normal diestrous intervals.

CYCLE IRREGULARITIES ASSOCIATED WITH OVARIAN PATHOLOGY

Gonadal Dysgenesis

Gonadal dysgenesis refers to the congenital lack of development of the ovaries. Only a "streak" (small, almost cylindrical) gonad is present, with no follicular activity. The remainder of the tract is intact, as the female tract will form in the absence of gonads, but the tract is juvenile because no ovarian steroids are present to stimulate growth. This abnormality is most commonly associated with defects of the X chromosome, including XO (Turner's syndrome) and XXX; however, it may be seen in XY mares as well as in mares with apparently normal XX karyotypes.

Mares with gonadal dysgenesis may be presented for examination because of anestrus, erratic estrus, or constant estrus. Exhibition of estrous behavior is due to the lack of progesterone. In cases of erratic or constant estrus, the mares may have been bred repeatedly without conceiving. Mares with XO karyotypes may be unthrifty, be shorter than anticipated, or have increased angulation of the hind legs.

Diagnosis of gonadal dysgenesis is based on history, karyotype, and repeated palpation and ultrasonography or progesterone determination. Mares with gonadal dysgenesis have never foaled and have never been pregnant. Palpation and ultrasonography reveal very small or apparently absent ovaries; if ovaries are present, no follicular activity is seen. It should be noted that some normal mares, especially young mares of smaller breeds, can have very small ovaries that function normally (raise follicles and ovulate with normal fertility). For this reason, serial examinations or progesterone determinations at least once weekly for 5 or more weeks during the breeding season may be needed to definitively state that the ovaries are nonfunctional. Presence of nonfunctional ovaries does not necessarily mean that the mare has gonadal dysgenesis: small, inactive ovaries during seasonal anestrus are normal; very old mares (>24 years) may have inactive ovaries in the breeding season because they have stopped cycling altogether; and mares having a severe energy imbalance due to starvation or disease (or, theoretically, mares in extremely hard work) may have inactive ovaries. A chromosomal abnormality on karyotype supports the diagnosis of gonadal dysgenesis. No treatment is possible for mares with gonadal dysgenesis.

Granulosa Cell or Other Functional Tumor

Granulosa cell tumors (GCTs) are by far the most common ovarian tumors in mares. These neoplasms of the sex-cord stroma may also contain thecal cells (granulosa cell–theca cell tumors). They may be found in mares of any age, and are almost invariably benign.

Behavioral changes associated with GCTs include aggressive or stallion-like behavior, constant or erratic estrus, or anestrus, with about equal frequency. These changes are attributed to steroid production by the neoplasm. Theoretically, production of testosterone or high concentrations of estrogen may be associated with aggressive or stallion-like behavior; estrogen with constant estrus; and progesterone with constant diestrous-like behavior; however, progesterone is typically low in cases of GCT. Anestrous behavior (including erratic signs of heat) may be associated with neoplasms that do not produce steroids. The contralateral ovary is typically inactive and small. This is attributed to high concentrations of inhibin or steroids from the tumor, causing suppression of follicle-stimulating hormone (FSH) release. In rare cases, apparently no pituitary suppression is present and mares with GCT cycle normally, ovulating from the contralateral ovary.

Diagnosis of GCT is based largely on the history (abnormal behavior or cyclicity) and findings on palpation and ultrasonography per rectum. The affected ovary is typically large, smooth walled, and spherical. The ovulation fossa is usually not present. The contralateral ovary is usually small and inactive, resembling an anestrous ovary. Ultrasonography is useful for differentiating normal follicular activity from pathologic changes in the ovarian stroma and for confirming the lack of activity of the contralateral ovary. There is no one typical appearance of GCT on ultrasonography, however. The most common ultrasonographic appearance is that of multiple cystic structures ("honeycomb" appearance); however, GCT may also appear as unilocular cysts or may be solid throughout.[6] The honeycomb appearance of a GCT may be confused with that of a clotting hematoma (see following discussion). A hematoma may be differentiated from a GCT by several features: with a hematoma, the activity of the contralateral ovary is normal and the mare has normal cycles; the ovulation fossa is present on the ovary, as the hematoma causes enlargement of only one pole; and the hematoma appears abruptly and regresses over time (usually within 30 days). Serum testosterone concentrations are elevated in 50% to 90% of GCT, and inhibin concentrations are elevated in over 85% of mares with GCT; tests for these hormones may be used as a diagnostic aid.[7] Management is by surgical removal of the affected ovary; approximately 75% of mares resume normal cyclicity from the remaining ovary within 2 years of removal of a GCT.

Teratomas are relatively rare in comparison with GCTs; they may be difficult to diagnose as the ovarian enlargement may not be pronounced, and ultrasonographic findings may be unremarkable. Although not commonly considered to be functional tumors, teratomas may be associated with disruption of cyclicity. Serous cystadenomas of mare ovaries have also been reported; these are associated with multiple large cystic structures resembling normal follicles on palpation and ultrasonography. Serous cystadenomas may be associated with elevated serum testosterone concentrations, but do not appear to affect cyclicity.

INTERSEXUALITY

Conditions in which testicular tissue is present in the gonad such as true or pseudo-hermaphroditism affect cyclicity but usually are suspected early in life because of intersexuality of the external genitalia. However, conditions may occur in which testicles are present but the external genitalia is that of a normal female (some XY sex reversals, including testicular feminization syndrome). These are associated with presence of internal testicles instead of ovaries, absence of cyclicity, stallion-like behavior, and usually failure of formation of the internal female tract, resulting in the vagina ending in a blind pouch. These conditions may be diagnosed on the basis of high serum testosterone levels, abnormal size, shape, consistency, or ultrasonographic appearance of the gonads, and abnormalities of the internal genitalia.

ABNORMALITIES OF OVULATION

Failure of normal ovulation of the dominant preovulatory follicle may occur in mares. This is identified on repeated ultrasonographic examination. Two abnormalities of ovulation have been noted clinically: anovulation with hematoma formation, and apparent failure of the follicle to completely empty at ovulation. The former abnormality has been documented but the latter has only been reported anecdotally among clinicians. No information on the pathogenesis of either abnormality has been reported.

In anovulation with hematoma formation (persistent anovulatory follicles), the preovulatory follicle appears to grow normally, but instead of ovulating, starts to rapidly enlarge. Whereas normal follicles typically grow about 3 mm per day, anovulatory follicles may suddenly increase 10 mm or more in diameter per day, becoming much larger than a normal preovulatory follicle. Echogenic particles become visible within the follicle, and may increase in number over a few days until the follicle may appear to be diffusely echogenic. At this time the contents of the follicle are fluid, and can be seen to swirl on ultrasonography when the follicle is balloted. Aspiration of the follicular contents at this time yields bloody fluid, and so these follicles are considered to be hemorrhagic. Finally, in the majority of anovulatory follicles, the contents of the follicle appear to clot (nonechogenic spaces form, delineated by strands of echogenic material).[6]

Increasing echogenicity over time and apparent clotting, resulting in formation of a hematoma, is not pathologic in follicles that would not normally be destined to ovulate (e.g., diestrus follicles, secondary follicles during estrus, dominant estrus follicles during fall transition [autumn follicles]). Anovulation of the dominant preovulatory follicle during the breeding season is less common and is associated with infertility. A 4.7% incidence of hematoma formation was found in one study,

but it was unclear how many of these were seen in mares that successfully ovulated another follicle at the same time.[1] In a second study, 8% of cycles in mares of varied reproductive history resulted in anovulation. This proportion was age dependent, being 4% in mares 6 to 10 years of age and 13% in mares 16 to 20 years of age.[8]

When anovulation occurs, more than 84% of mares progress into diestrus with progesterone concentrations greater than 1 ng/ml.[8] In these mares, the wall of the follicle surrounding the hematoma appears to thicken, suggesting that it is luteinizing. In one study all such structures responded to prostaglandin administration, but the day of administration was not given.[8] Clinically these luteal structures may appear not to respond to prostaglandin as readily as do normal corpora lutea, and repeated doses of prostaglandin may be needed to cause regression of the structure and return of the mare to estrus. Formation of an anovulatory follicle is associated with failure of conception in inseminated mares.[8]

Mares that experience anovulation and hematoma formation of the dominant follicle tend to do this over repeated cycles.[8] The author observed one Warmblood mare that formed a hematoma instead of ovulating at each estrus, throughout a breeding season. Treatment with human chorionic gonadotropin (hCG), suppression of follicular activity with progesterone and estrogen with subsequent release, and short-cycling with prostaglandin had no effect on hematoma formation. The mare was bred on each estrus but did not get pregnant. The next year, she was bred on an early estrus, ovulated normally, and became pregnant.

Mares experiencing anovulation/hematoma formation of the dominant preovulatory follicle may in fact appear to cycle normally, due to luteinization of the follicle wall. Some cases may be associated with prolonged diestrus; however, the presenting complaint may be simply infertility. Diagnosis entails monitoring follicle growth during estrus daily by ultrasound examination. Findings are those for normal follicle growth up to about ovulatory size (35–40 mm); then these follicles continue to grow, often rapidly, sometimes to a large size (60–90 mm). Echogenic particles usually are seen at the time of follicle enlargement. The echogenicity increases, then apparent clotting occurs. No specific causes or treatment for this syndrome are currently known.

Apparent incomplete emptying of the follicle at ovulation is much less well documented, but has been noted by practitioners. The follicle grows normally and then is seen to be partially collapsed, suggesting that the examination caught the follicle in the process of ovulation (this in fact would be a rare occurrence, as normal ovulation results in follicle emptying within about 5 minutes). Examination the following day shows the follicle to have an unchanged appearance, (i.e., to still be "ovulating"), irregular in outline and containing 15 to 25 mm of fluid within it. This appearance is maintained over the following days, then the fluid regresses slowly. The follicle wall appears to luteinize, and progesterone levels during the ensuing diestrus may be normal. While no hard data is available, the clinical impression is that the pregnancy rate for mares having this follicular appearance at ovulation is low. Again, no specific causes or treatment for this syndrome are known.

FOLLICLE GROWTH AND REGRESSION WITHOUT OVULATION

Follicle growth followed by regression, without ovulation, during the breeding season is extremely rare, but may occur. It is difficult to find documentation of this phenomenon, but it is recognized clinically. The author has observed this syndrome in two 14-hand, 5- and 10-year-old ponies, both pintos. The ponies were obtained from a dealer without knowledge of previous breeding history. Ovarian activity was followed throughout the breeding season by ultrasonography at least three times weekly. The ponies had apparently normal reproductive tracts on palpation and ultrasonography, and the ovaries were of normal size. Multiple follicles smaller than 10 mm were present, and periodically, a follicle would grow to 18 to 20 mm, only to gradually regress. Treatment with prostaglandin to lyse any luteal tissue present, and administration of hCG at peak follicle growth had no effect. No karyotype was done on these mares; however, because of the normal ovarian size and follicle growth, they could not be classified as having gonadal dysgenesis.

BEHAVIORAL PROBLEMS ATTRIBUTED TO THE OVARIES

Persistent Estrus

"Persistent estrus" is a fairly common complaint in working and racing mares. The major factor in the complaint is that the mare is constantly showing a behavior, interpreted as estrus, and it is interfering with the mare's function. Upon investigation, this complaint is usually not associated with any actual abnormality in cyclicity, and it is seldom identified in mares in a teasing program where true estrous behavior (response to a stallion) can be assessed.

When evaluating histories of persistent estrus, the normal variation of estrous length, from 2 to over 10 days, should be considered. The pattern of estrous cyclicity noted by the owner, and exactly what behavior the owner is seeing, should be ascertained. A major confusing issue in these mares is what is considered to be estrous behavior. Such mares may be characterized by their owner as being irritable, kicking when their sides are touched, leaning on the handler, striking, urinating, or wringing their tails. These signs are interpreted as indicating that the mare is in heat; however, they may be simply signs of agitation, discomfort, or submission.

To diagnose the cause of the apparent constant estrous behavior, findings on palpation and ultrasonography of the tract are evaluated with the history, and the mare should be teased with an active stallion. Response of the mare to the advances of the stallion with signs of estrus, such as urinating, raising the tail, winking the vulva, and hesitating to move away from him, accompanied by estrus-type signs on palpation and ultrasonography as outlined earlier, indicate that the mare is in estrus at the

time of the examination. Mares with large follicles (>33 mm) may respond to administration of hCG (2500 IU IV) by ovulating approximately 2 days later and going out of heat in another 1 or 2 days. If monitored, mares in estrus with small follicles will usually show normal follicle growth, ovulation, and entrance into diestrus. The reason for the history of prolonged heat in these mares before the examination is not clear.

Constant or erratic true estrous behavior (response to a stallion) may be seen during seasonal transition (see previous discussion). This behavior may also be related to ovarian pathology such as gonadal dysgenesis or granulosa cell and related tumors, and these possibilities should be investigated as outlined earlier. In addition, pregnant mares may show constant or irregular estrus in response to teasing by a stallion.

In a nontransitional mare with a normal nonpregnant tract showing persistent behavior interpreted as heat, any ovarian basis for the behavior may be determined by establishing whether there is a correlation between behavior and cyclicity. The reproductive tract should be examined two to three times weekly for a month or more while the owner notes the mare's behavior. If the mare cycles normally, and no correlation of cyclicity with behavior is present, the mare's behavior is probably not associated with her ovaries.

Many nonreproductive behaviors may be interpreted as signs of "heat." Mares that have constant agitated behavior, rather than true estrous behavior, may be in any stage of the cycle at the time of examination. Agitated, estrous-type behavior is often seen (or at least complained about) in mares in fairly intense training. This behavior has been termed "submissive, cowering behavior" and has been equated to the urinating behavior of a scared puppy.[9] When mares with this behavior are evaluated critically, although some signs associated with estrus may be present (raising the tail, urinating, leaning), the behavior of the agitated mare is anxious, or guarding.[9] She is resentful of the approach of a stallion when teased, and may lean away from the stallion or appear fearful and attempt to escape. This behavior is in contrast to that shown by the mare in true estrus, which during teasing is typically calm and interested in maintaining contact with the stallion. Mares with cowering behavior may improve given changes in their environment and gentle handling, with the goal of increasing the mare's confidence and decreasing intimidating stimuli while reacclimating her to her training schedule. Long-acting tranquilizers have also been felt to aid in management of these mares.[10]

Signs interpreted as persistent estrus may be attributable to vaginal inflammation due to a foreign body, or to aspiration of air into the vagina when the mare is working. The latter seems to be most common in racing-fit mares (mares with little body fat) in training. These mares lack perineal fat, which affects the tone of the vulvar lips, the angle of the vulva, and the weight of the perineal body, which closes the entrance to the vagina. The affected mare has signs of vaginitis such as frequent urination, hunching the back, dragging the hind feet, and wringing the tail. Racing mares with this complaint commonly have a history of "stopping" part way through training periods; this may be due to pneumovagina incurred during work. Examination of the tract shows the mare in any stage of the cycle; bright echogenic particles representing air may be seen in the uterus on ultrasonographic examination; the vagina may be ballooned with air. Speculum examination of the vagina may reveal an inflamed vagina and the mare may show extreme signs of irritation (hunching the back, attempting to urinate) after the speculum is inserted or removed. This problem may occur even after a Caslick's operation has been performed. A modified Caslick's procedure (perineal reconstruction) may help to prevent pneumovagina by narrowing the vestibule and increasing the bulk of the perineal body.

Abnormalities of the bladder or urethra, such as cystitis or urethral masses, may result in frequent urination that is interpreted as persistent estrus. One mare, presented for examination for "nymphomania," exhibited agitated, pacing behavior, and frequent urination. The cause was found to be neurogenic bladder atony with associated cystitis. The distended bladder constantly ejected urine as the mare moved. When the bladder was kept empty by catheterization, the mare's constant tail raising, posturing, and agitated behavior all ceased; the agitation may have been due to pain from bladder distention. The mare's reproductive tract and cyclicity were normal.

Sometimes stallion-like behavior is interpreted to be estrous behavior by the owner. Assessment of stallion-like behavior may be done by turning the mare out with other mares and observing whether she exhibits courting or mounting behavior, by placing her in a stallion's paddock to observe how she responds to fecal piles (stallion-like behavior includes smelling fecal piles, defecating or urinating on the same pile, and smelling the pile again), and by bringing mares up to the fenceline to see if the mare under study prances up, vocalizes or otherwise behaves in a stallion-like manner. Stallion-like behavior is almost always related to exposure to androgens, possibly from an ovarian tumor or presence of testicular tissue (see previous discussion) or from administration of exogenous anabolic steroids. The effects of exogenous androgens may last for months after the last administration.

Cyclic or Sporadic Behavioral Problems

Some mares do become harder to manage, perform irregularly, or even appear lame when in heat; this behavior is intermittent and corresponds to specific stages of the estrous cycle.[9] Such problems may be due simply to exaggerated exhibition of normal estrous behavior at times that are deemed inappropriate by the owner (e.g., while under saddle). However, there may also be behavioral and physical effects of estrogen, such as increased joint laxity, that affect performance. Some part of this behavior may also be related to pain from the growing follicle stretching the ovarian tunic, or from rupture of the follicle at ovulation. Most mares exhibit signs of pain when the ovary is palpated immediately after ovulation.

Altrenogest treatment (at 0.044 to 0.132 mg/kg orally daily) is commonly used to suppress problem estrous behavior in mares, and is usually effective. Although

progestin-containing implants produced for estrus synchronization in cattle have been used for this purpose, and anecdotally have had some effect, studies have shown that most synthetic progestins do not suppress estrus or interact with equine progesterone receptors. Sometimes owners request ovariectomies for mares that are sporadic or constant "bad actors." This should be reserved only for cases in which the mare has *cyclic* behavioral problems corresponding to a specific part of the estrous cycle when evaluated as described earlier; this behavior should seem to improve over the winter (during anestrus). Mares rarely fall into this category, but when they do, ovariectomy can be an effective cure for the behavior. The mare's tendency to react violently should be considered when making the decision on whether to do the ovariectomy standing (via colpotomy) or under general anesthesia.

As with "persistent estrus," however, owners may attribute objectionable behavior in a mare to ovarian problems when in fact there is no connection. Any type of sporadic behavior (even refusing jumps when under saddle) may be interpreted by the owner as a sign that the mare is in heat. Owners may assign a behavior to 21-day cycles because they are associating it with estrus, and they are aware of the length of the estrus cycle; however, questioning may reveal that the actual duration of the behavior and the period between behavioral episodes is unknown. These cases should be approached with the presumption that the behavior is not related to cyclicity until the association is proved. Examples of this include a mare that was presented for examination with a history that she was in pain during estrus, showing signs of colic and inappetence. The owners had not kept a written record but stated that the episodes occurred about every 21 days. A work-up for recurrent colic was performed, and on gastroscopy the mare was found to have stomach ulcers. Treatment of the ulcers with cimetidine resulted in cessation of the colicky episodes. In another case, a mare was periodically resentful of being handled, to the point of shaking and attempting to lie down; complete examination, including exploratory laparotomy, revealed an abscess in the stomach wall.

Aggressive or Abnormal Behavior

Constant aggressive, quasi-stallion-like or otherwise abnormal behavior may be related to presence of a granulosa cell tumor or testicular tissue (see previous discussion). Interestingly, it appears that some mares may show changes in behavior for a year or more before the detection of ovarian changes indicative of GCT.[10] Therefore, if the reproductive tract is normal on initial examination, but no other cause of abnormal behavior is documented, a repeat examination of the reproductive tract should be performed every 6 months or so.

A warning should be made regarding mares presented for assessment of ovarian causes of abnormal behavior. Occasionally a mare is presented that has vicious outbursts of aggressive behavior—in one case that the author evaluated, the mare broke through a fence to attack a child. These mares may be presented for reproductive examination on the possibility that violent or vicious behavior is related to an ovarian abnormality. Such mares should be approached with caution. Little information is available on the causes of this behavior but it is unlikely to be related to the reproductive tract. In two cases of such behavior seen by the author, pituitary abnormalities were suggested. One mare had preneoplastic changes in the pituitary on histopathologic examination at necropsy, and one mare had adrenocorticotropic hormone (ACTH) concentrations and dexamethasone suppression test results midway between those expected for normal and for Cushing's disease patients.

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CHAPTER 19

Infertility Due to Noninflammatory Abnormalities of the Tubular Reproductive Tract

HEIDI IMMEGART

Infertility in mares can result from developmental abnormalities or acquired conditions that cause reproductive failure due to inflammation with or without infection in the reproductive tract, abnormal physiologic events, abnormal reproductive tract anatomy, structural alterations resulting in abnormal function, and neoplasia. This chapter will focus on infertility that is due to congenital or acquired problems of the tubular reproductive tract that are noninflammatory in nature.

REPRODUCTIVE TRACT DEVELOPMENT

Initial development of the reproductive tract is determined by the animal's genetic sex, which is responsible for stimulation of gonadal differentiation. Normal testis determining factor (TDF) genes on the Y chromosome along with the H-Y antigen complex cause development of testes with seminiferous tubules in the undifferentiated gonad of the normal XY male. Sertoli cells within the testis produce müllerian inhibiting substance (MIS), which suppresses development of the paramesonephric (müllerian) ducts while testosterone production from the Leydig cells of the developing testis stimulates development of the mesonephric (wolffian) ducts into the epididymis and vas deferens. Testosterone also stimulates masculinization of the external genitalia following conversion into dihydrotestosterone in those tissues.

The absence of the Y chromosome along with genes on the X chromosome results in differentiation of the fetal gonad into the ovaries in the normal XX female. Without testosterone and MIS, the wolffian ducts regress and the müllerian ducts develop into the female tubular reproductive tract. The nonfused cranial portion of the paired paramesonephric ducts forms the oviducts and uterine horns. The caudal portions of the paramesonephric ducts fuse and develop into the uterine body, cervix, and vagina. The absence of testosterone, and thus dihydrotestosterone, results in feminization of the external genitalia and development of the vulva, clitoris, vestibule, and caudal vagina.

Abnormal reproductive tract development can arise from an abnormal chromosomal/genetic condition or an abnormal hormonal environment as well as from exogenous factors.[1] Many different conditions that result in abnormal development of the reproductive tract in the horse have been reported. The most common genetic condition is X monosomy or (63XO) gonadal dysgenesis, which is the presence of an aneuploid defect of the sex chromosomes.[2] The fetus develops into a female with hypoplastic ovaries and a small underdeveloped tubular reproductive tract with hypoplastic endometrial glands. These animals fail to cycle normally even following hormonal treatment and are considered sterile. Attempts to establish pregnancies via embryo transfer into these mares have not been successful enough to consider these animals appropriate recipients. Affected mares are typically small and weak at birth and remain small in stature as adults. The condition is thought to arise from nondisjunction during meiosis and is sporadic in nature rather than hereditary.

The second most common condition is XY sex reversal.[3] XY gonadal dysgenesis results in a gonadal female with ovaries or streak gonads and a variability in female phenotype from normal to one with hypoplastic or aplastic tubular genitalia. These animals fail to demonstrate normal cyclic estrous behavior despite hormonal stimulation. This author has seen one Thoroughbred mare with the XY gonadal dysgenesis syndrome. This mare would sporadically display estrous behavior, presumably from adrenal steroid production.

The XY testicular feminization condition is associated with a lack of conversion of testosterone to dihydrotestosterone or an insensitivity to dihydrotestosterone. This results in feminization of the external genitalia concurrent with a lack of development of the internal female tubular reproductive tract. The gonads of these animals may contain both testicular and ovarian tissue and behavior is frequently masculinized. XY sex reversal may in some cases be caused by a single gene defect passed through the male or female on the X chromosome.

A multitude of other genetic/chromosomal anomalies have been reported in conjunction with hermaphroditic, pseudohermaphroditic, and subfertile conditions including XX sex reversal, XXY, mosaicism, and chimerism. These animals have displayed varying degrees of gender

ambiguity, associated with the internal and external portions of the reproductive tract, and infertility.

Other developmental abnormalities will be discussed with regard to the structure that is affected.

Oviducts

The oviduct of the mare consists of the infundibulum, which is closest to the ovary, the ampulla, and the isthmus, which is adjacent to the uterine horn. Fertilization occurs in the ampullar region and fertilized ova are transported into the uterus. Unfertilized ova from cycles tend to remain in the oviduct.

Congenital Defects

Although rare, developmental abnormalities involving the oviducts have been reported.[4] Segmental aplasia is the condition in which a portion of the oviduct does not develop. Hydrosalpinx may result from the lack of patency of the oviductal lumen depending on the location of the aplastic segment. Some cysts or series of cysts near the caudal portion of the oviduct are thought to be a persistence of accessory oviducts that may develop at the end of the paramesonephric duct. Large cystic structures may be a cause of infertility.

Acquired Abnormalities

Acquired abnormalities of the uterine tube are usually secondary to disease processes occurring around other structures. For example, adhesions involving the oviduct may develop secondary to salpingitis, which typically arises from severe inflammatory/infectious processes in the uterus or abdomen. Adhesions that obstruct the lumen from external pressure may also cause hydrosalpinx.[4] Fertilization following ovulation from the ipsilateral ovary would be prevented in cases in which complete occlusion of the oviductal lumen is present.

Although globular collagenous masses have frequently been reported to occur mainly in the ampullar isthmic junction area, the clinical significance of these structures remains controversial. Unfertilized oocytes have been located on the up- and downstream aspects of many of these masses, and large collagenous accumulations appeared to fill and in some cases distend the oviductal lumen. Oviductal blockage could prevent oocyte/embryo migration past the mass. One study demonstrated increased pregnancy establishment following surgical intervention to re-establish oviductal patency of previously blocked tubes in a group of mares with a 2- to 4-year history of infertility.[5] Although not easily diagnosed under field conditions, oviductal occlusion should be a consideration in mares in which other causes of infertility have been ruled out.

Sperm capacitation and hyperactivation, fertilization, and early embryonic development all occur within the oviduct. It is possible that functional or secretory abnormalities may affect one or more of these critical steps. Protein secretion patterns have been determined to be different between oviducts from young fertile mares as compared to aged infertile ones. Epithelial cysts in the ampullar region and adhesions in the infundibulum have been described.

Uterus

Developmental Defects

Developmental abnormalities of the uterus include hypoplastic conditions as well as segmental aplasia and uterine body duplication. Hypoplasia of the uterine body and horns is commonly associated with the XO gonadal dysgenesis and XY sex reversal as well as other chromosomal anomalies.

Segmental aplasia is the condition in which a portion of the uterine horn or body does not develop. This author has observed cases in which aplasia has involved one entire uterine horn. The mares involved had a history of being difficult to get in foal and subsequently bearing small offspring. Two other mares were reported to establish pregnancies when the ovary ipsilateral to the existing horn had ovulated.[6] Segmental aplasia involving one uterine horn prevents sperm from contacting the ovum following ovulation from the ovary of the aplastic side. When the aplastic segment includes the uterine body, sperm are completely prevented from passage into the oviduct and fertilization. Depending on the location of the aplastic segment in one uterine horn, this condition may prevent adequate uterine migration by an embryo conceived from an ovulation by the contralateral ovary. Inadequate uterine migration by an embryo will result in a lack of maternal recognition of pregnancy. Decreased functional uterine space may also limit placental growth and result in smaller offspring, premature delivery, or abortion. One mare with repeated abortions was reported to have a short or absent uterine body.[7] Pregnancy loss was attributed to insufficient placental surface.

Uterine body duplication occurs when the bilateral paramesonephric ducts fail to fuse normally. Varying degrees of this abnormality have been observed, ranging from a short septum located at the caudal portion of the uterine body to a complete septum dividing the entire length of the uterine body to the cervix. This aberrant fusion may even involve the cervix as well. One mare was reported to have had a complete division of the uterine body with a separate cervix for each side and a dorsoventral curtain present in the cranial vagina. Lack of sperm transport to the appropriate oviduct and insufficient uterine migration or functional space may be associated with infertility in animals with this abnormality.

Endometrium

The inner lining of the uterus is the endometrium. Within the endometrial epithelium are glands that secrete nutrients necessary for normal embryonic and fetal development. Histologic evaluation of the endometrium of mares has demonstrated changes in the development and secretory nature of the glandular epithelium as well as changes in the luminal epithelium, other cell populations, molecular components, and steroid receptor expression of the uterine lining consistent with normal estrous cyclicity.[8] The changes detected are synchronous with the changes in the hormonal environment encountered during the estrous cycle. The changing local environment of the endometrium at different stages of the cycle has been associated with essen-

tial processes that occur at specific times such as sperm functional changes, embryonic support, fetal support, and placental development. Abnormalities of the endometrium due to abnormal development, secretory function, synchrony, or cellular composition as well as degeneration have been associated with infertility.

Endometrial hypoplasia is the lack of normal development of the endometrial glands. This condition is recognized in association with abnormalities such as gonadal dysgenesis as well as with the normal situation prior to puberty. With puberty and the first ovulatory cycle, endometrial glands differentiate into the adult secretory structures. It has been determined that estrogen is required to prime the uterus for progesterone stimulation of glandular differentiation.[9] With repeated hormonal stimulation associated with normal estrous cycles, the endometrial glands proliferate such that they can provide adequate support to a pregnancy; however, some young mares have been noted to have delayed endometrial maturation and have been categorized as hypoplastic. Increased pregnancy loss rates are observed in 2-year-old fillies and have been associated with the lack of glandular development required to support gestation to term.

Glandular atrophy is the condition in which the endometrial glands, while normal in development, at some point in time are lost.[5] This occurs naturally during seasonal anestrus; however, return to normal endocrine activity may not correlate with return to cyclicity. Endometrial atrophy in cyclic mares can be a cause of early embryonic death. The condition is also observed in older mares and mares undergoing ovarian senescence. Atrophy can be caused by a lack of ovarian steroids. Treatment is not usually productive. Endometrial atrophy and hypoplasia may be difficult to distinguish, however, and history of previous endocrine function and reproductive tract evaluations can help differentiate the two.

Glandular hyperplasia has also been described in mares as a cause of infertility. The hyperplastic condition has been associated with delayed involution following fetal loss or abortion.[10] It can also be seen in conjunction with steroid secreting tumors such as granulosa cell tumors and is reversible in these cases following tumor removal.

Endometrial maldifferentiation has been studied in barren mares. Several patterns of irregular or unequal differentiation of the glandular structures and secretory patterns that did not correspond appropriately with the stage of the estrous cycle from which the biopsy sample was obtained were classified.[11] The prognostic value of these maldifferentiation categories and the abnormal secretory patterns associated with some of them are under investigation. Maldifferentiation is known to cause infertility in other species such as the human. It has also been demonstrated in some species that abnormal endometrial development and glandular differentiation and secretion can be altered if the receptors for the stimulating hormones are deficient or absent.[1,12] In these cases, females undergoing pubertal changes in the hormonal environment do not have concurrent maturation of the uterine lining. Infertility is detected in these individuals. Genetic alterations and chemical encounters at various stages of development have been associated with these cases.

Endometrosis is a syndrome encompassing a group of degenerative changes, typically including periglandular fibrosis, cystic dilation of glands, and glandular necrosis or hyperplasia of the endometrium. These degenerative alterations have long been associated with infertility which may result from damage to the endometrial surface, changes in histotroph components or secretion, retardation of embryonic and fetal development, and delay in placental development. Glandular protein production has been demonstrated to be deficient in some and secretion to be altered, indicating glandular asynchrony, in other mares with endometrosis when compared to normal fertile mares. The pathogenesis of endometrosis is not completely understood; however, associations between the presence of endometrial degeneration and angiotensin-converting enzyme, an enzyme associated with fibrosis in tissues including heart, kidney, liver, and spleen, have been detected in the mare as well as modulations in fibroblasts and collagen IV. This condition has been considered an age-related change that has no consistently functional treatment or prevention in mares.

Microscopic changes of the blood vessels in the endometrium have been noted in aged and multiparous mares. Sclerotic changes have been detected in aged maiden mares and a syndrome similar to pregnancy sclerosis of other species has been detected in multiparous mares.[13] The changes noted included elastosis and fibrosis of the intimal media and adventitial layers. Alterations in endometrial blood flow have been associated with repeated pregnancy failure in older mares.

Endometrial cysts are structures seen typically in mares over 10 years of age and are either glandular cysts or lymphatic lacunae.[8] Glandular cysts frequently are associated with periglandular fibrosis resulting in fibrotic nests that may become just large enough to detect with ultrasonographic examination. Some glandular distentions have been noted without fibrotic change and also have been associated with infertility when the condition persists during the physiologic breeding season. Lymphatic lacunae are dilated lymphatic vessels. These may coalesce and become much larger than glandular cysts and have glandular atrophy associated with the epithelium covering the area. These cysts are typically located at the base of the uterine horns and have been associated with higher biopsy categories. Although single smaller cysts may not affect fertility greatly, large or numerous cysts have been associated with reduced fertility in mares, possibly from increased early embryonic death, reduced embryo migration, and increased embryonic resorption or abortion. Ultrasonography can be used to diagnose the presence of cysts; however, hysteroscopy can be both diagnostic and therapeutic, via cyst ablation.

Changes in the endometrium of mares can be readily detected by histopathologic examination of a biopsy sample. Typically, one sample will be representative of the entire uterus due to the diffuse nature of most abnormalities; however, some focal changes in endometrial structure have been detected. Lymphatic lacunae tend to be localized and are not readily diagnosed with biopsy,

and a decrease in glandular structures has been reported near the internal cervical os. Multiple biopsies may be beneficial if a single tissue sample has been inconclusive or if it is suspected that a lesion is localized or affected by season. The significance of uterine biopsy sampling cannot be overemphasized in aiding the diagnosis of mare infertility.

Uterine Lumen

Adhesions involving the lumen of the uterus may obliterate the glandular surface as well as cross transluminally as bands or sheets. Gross scar tissue formation is typically the result of uterine trauma from dystocia, severe inflammation or infection, or inappropriate administration of caustic substances. Transluminal sheets may cause accumulation of uterine secretions cranial to the blockage. Pyometra may result if the fluid becomes contaminated. Scar tissue may be visible with ultrasonographic examination of the uterus in cases in which fluid accumulation is detected or hyperechoic mottling is present in the lumen. Endoscopy may be used to visualize the presence and degree of adhesion formation or may be blocked by the presence of transluminal sheets. Prognosis for fertility depends on the extent of the adhesions. Although minimal bands may be broken down via endoscopy, generalized uterine insult tends to result in adhesions that are diffusely distributed throughout the uterus.

Mucometra is the abnormal accumulation of mucus in the uterus. As a primary condition, mucometra is rare in mares and is associated with cystic dilation and lymphocytic infiltration.[4] Mucometra may be secondary to the presence of a complete hymen in young cycling mares or cervical adhesions. Unbreached, the mucus remains sterile inside the reproductive tract; however, prolonged exposure to large volumes of mucus may eventually alter endometrial structure or function.

Uterine Wall

Uterine neoplasia is rare in the horse. Tumors reported in equine uterus include benign tumors such as leiomyomas, fibromas, and fibroleiomyomas, as well as malignant tumors such as leiomyosarcomas, rhabdomyosarcomas, lymphosarcomas, and adenocarcinolas.[14] The most common of the tumors reported is the leiomyoma, which is a benign neoplasm derived from the smooth muscle. Neoplasia can be diagnosed utilizing transrectal palpation and ultrasonographic imaging, intrauterine endoscopy, and biopsy. Removal of the tumor mass is indicated for mares being bred; however, large or invasive tumors may require hysterectomy.

Uterine wall hematomas and hemorrhage into the lumen of the uterus typically arise at delivery and usually resolve given time.

The abnormal location of the uterus, tilting ventrally in relationship to the pelvis, has been associated with a lack of normal uterine clearance.[15] Decreased capacity to clear the uterus of fluid and debris results in decreased fertility. Increased uterine clearance is evident following oxytocin treatment of both normal mares and mares with decreased uterine clearance. Ventral sacculations of the uterus, located at the uterine horn and body junction, have also been associated with infertility. These are seen in infertile older mares and may be focal areas of mucosal atrophy or myometrial atony, which accumulate bacteria, fluid, and debris.[16] Treatments including postbreeding oxytocin have been used with variable results, and uterine lavage remains controversial.

Cervix

Developmental Defects

Cervical hypoplasia is the condition in which the cervix is underdeveloped. This has been reported in one mare with normal ovarian activity; however, it is more frequently seen with conditions such as XO gonadal dysgenesis as well as other genetic/chromosomal abnormalities. Typically other reproductive tract anomalies exist concurrently. Cervical hyperplasia was reported in one mare with tissue protruding through the vulva; however, it was not determined whether the benign mass was congenital or acquired.[17] The mare was also noted to have ventral sacculations of the uterus and endometrial atrophy. Double cervix is the condition in which fusion of the caudal paramesonephric ducts is not completed. This condition may exist alone or in conjunction with uterine body duplication or dorsoventral vaginal curtains. Both, or only one, of the cervical canals may be patent. Congenital absence of the cervix through segmental aplasia of the paramesonephric duct is rare in the horse, but it was reported once in a pony mare.[18] Accumulations of uterine secretions were evident due to complete blockage of outflow at the aplastic segment.

Acquired Abnormalities

The cervix is a dynamic organ that must be able to close tightly in order to protect the uterus and early pregnancy as well as relax appropriately during estrus and fetal delivery. Damage to the cervix typically occurs at delivery and may or may not be associated with dystocia. Cervical lacerations may be gross tears of the epithelial and muscular layers of the body or os or they may be microscopic breaches of the muscular layer only. Damage to the cervix may be inflicted by direct trauma at delivery or breeding or may be due to inadequate cervical relaxation for delivery of the fetal shoulders or pelvis. The latter type of trauma is frequently a result of forced fetal retraction prior to adequate cervical relaxation for normal fetal passage. Damage involving only the external os of the cervix may not hinder fertility if the cervical body is capable of achieving adequate closure for pregnancy protection. Gross or microscopic lacerations that render the cervix incapable of closure (cervical incompetence) will result in infertility. Cervical competency can be determined by digitally examining the cervix when the mare is under the influence of progesterone. Surgical repair of cervical lacerations has been attempted with variable results. Suture failure, scar tissue formation, and the potential for reinjury at subsequent foaling may confound treatment.

Cervical adhesions may also result from trauma to the cervix. These adhesions may be intramural or transluminal or they may extend from the external os to the cranial

vaginal wall. Scar tissue formation can result from trauma to the cervix or overwhelming uterine inflammatory disease. Depending on the location of adhesion formation, the cervix may not be capable of normal closure, resulting in infertility. Alternatively, the cervix may scar closed either by cervical wall or by transluminal adhesion formation. Fluid accumulation may be noted in these cases. The capacity of the cervix to close as well as luminal patency and the presence of scar tissue is most accurately determined by palpation of the cervix manually through the vagina. Gross adhesions may be broken down digitally or with long-handled scissors; however, recurrence typically follows.

Cervical neoplasia is rare in the mare; however, two recurrent leiomyomas were reported in half-sibling mares.[19] One fibroma, which interfered with normal function of the cervix, was removed and normal cervical competence was restored.[20]

Vagina

Congenital Defects

Persistent hymen in the mare is the condition in which the opening between the caudal vagina and the vestibule is not complete. A partial persistent hymen may present as a band or bands of tissue stretching across the opening of the vagina and typically has no effect on fertility. A complete hymen is a sheet of tissue that occludes passage into the vagina from the vestibule. These are typically diagnosed in young postpubertal mares in which fluid accumulation cranial to the hymen causes protrusion of the hymen through the vulva. Excision of the hymen tissue corrects the condition; however, chronic distention of the uterus may cause infertility due to endometrial atrophy, or bacterial contamination of the fluid may cause pyometra. One case of vestibular-vaginal hypoplasia was reported in a pony mare in which the caudal vaginal/vestibular area had a diameter of only 4 cm.[21] The cranial vaginal diameter was normal, and no evidence of trauma was apparent at the strictured area.

Acquired Abnormalities

Infertility due to vaginal abnormalities is most commonly associated with adhesion formation after trauma due to delivery or, less frequently, breeding accidents. Scar tissue formation within the vaginal canal can be extensive following dystocia and may nearly obliterate the vaginal canal. This author has seen two mares in which vaginal penetration was made impossible by extensive adhesions in the caudal vagina following dystocia.

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CHAPTER 20

Inflammation of the Tubular Reproductive Tract of the Mare

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A broodmare needs to produce consistently viable foals to be economically successful. In order to achieve this she needs to be in good physical condition, have regular estrous cycles, mate, conceive, maintain pregnancy, give birth, and raise a foal. If there is a breakdown in any of these areas, she will become considered a "problem" mare. Consideration of management practices and fertility of the stallion is therefore important before all the blame of subfertility or infertility is placed on the mare. The tubular part of the reproductive tract is composed of the vulva, vestibule, vagina, cervix, uterus, and oviducts. All work together to provide the most conducive environment for fertilization, embryonic development, and the birth of a healthy foal. Inflammation of one of these components affects the others.

VAGINITIS AND CERVICITIS

The mare's reproductive tract resides in the caudal portion of the abdomen suspended from the body wall by the broad ligaments. As the number of estrous cycles and foals produced increases so does the laxity of these ligaments causing the reproductive tract to lie more cranial and ventral to the pelvis. Three major anatomic barriers protect the uterine environment: the vulva, labia, vulvovaginal fold (hymen), and the cervix.[1,2] Fertility problems begin to arise in the mare when these anatomic barriers are compromised or fail. This leads to contamination of the reproductive tract by air, urine, or particulate matter, causing pneumovagina, vaginitis, cervicitis, and endometritis.

The vagina and uterus are believed by some investigators to have a normal bacterial flora considered to be nonpathogenic, whereas others assume that the uterus of the clinically normal mare is bacteriologically sterile.[3] Whichever the viewpoint, clinical vaginitis and cervicitis is most commonly due to irritation from air, urine, particulate matter (bacteria), or chemicals including antibiotics and antiseptics and are usually associated with endometritis.[4–6] The vulvovaginal fold, rather than the cervix, has been indicated as the major barrier to ascending bacterial contamination of the mare's reproductive tract.[3] Speculum examination reveals a hyperemic mucosa of the vaginal vault and cervix with or without exudate or urine crystals on the ventral aspect, depending on the cause. Treatment is focused on reconstructing the physical barriers to include Caslick's procedure, perineal body reconstruction, repair of perineal lacerations and rectovaginal tears, and urethral extensions.[1,4] These steps will remove the offending source of irritation or organisms allowing for the vaginitis and cervicitis to resolve. If the underlying problem is endometritis, intrauterine or systemic treatment with an appropriate antibiotic or discontinuation of uterine treatment of irritating antibiotics (enrofloxacin, ceftiofur) or antiseptics (Nolvasan, concentrated iodine solutions) resolves the secondary vaginitis as long as permanent damage such as adhesions has not occurred.[7]

Trauma to the vagina and cervix during breeding or parturition can also be a source of inflammation. Bruising of the mucosa of the vaginal vault and cervix can occur when the stallion is overzealous during mating, especially in maiden mares. This can be alleviated with the use of a breeding roll so that penetration can be controlled and restricted. During parturition, the foal's feet can damage the mucosa, or if assistance is necessary, chains and fetatomes can cause injury. Necrosis of the vaginal mucosa can occur if the trauma is excessive. Unfortunately, clinical observation but not documentation of this ailment has been made. The vaginitis or cervicitis produced will in most cases resolve with time.

ENDOMETRITIS

After fertilization occurs in the oviduct, it takes 5 to 6 days for the embryo to descend into the uterus.[8] Therefore, in order for the embryo to continue to develop, the uterus needs to provide a conducive environment for growth, free of inflammation and contamination. Endometritis is an inflammation of the endometrium of the uterus. It has been described as being the third most important clinical problem in equine practice after colic and respiratory tract disorders.[9] As previously described, endometritis is often seen in conjunction with vaginitis and cervicitis, when compromise to the physical barriers or contamination during breeding occurs,[10–12] and is associated with infertility in the mare.[13,14] Mares have been classified depending on their ability to clear their uterus in a certain amount of time when challenged with infection as susceptible or resistant.[15] Normal/resistant mares are able to clear their uterus from infection or inflammation by three mechanisms: uterine contractions, lymphatic drainage, and an open cervix.[16–19] Antibody-mediated uterine defense appears to be functional in susceptible mares, and although polymorphonuclear neutrophils (PMNs) are not dysfunctional, susceptible

mares were found to have impaired phagocytosis as a result of insufficient opsonization in uterine secretions.[10,20] The breakdown in uterine physical clearance mechanisms is currently believed to play a major role in susceptibility to persistent endometritis.[16] Further identification of these mares has been established by using scintigraphy and ultrasonography in which impaired or reduced myometrial contractility in response to acute inflammation results in an accumulation of fluid and inflammatory products within the uterine lumen.[10,21–24] Additional factors including vascular degenerative changes and dependent position of the mare's uterus have also been implicated in interfering with effective clearance of the uterus.[10,21,25]

Based on the current literature, persistent endometritis can therefore be divided into (1) sexually transmitted diseases (STDs), (2) chronic infectious endometritis, (3) persistent breeding-induced endometritis, and (4) chronic degenerative endometritis (endometriosis).[10]

SEXUALLY TRANSMITTED DISEASES

Sexually transmitted diseases are acquired after mating mares with stallions that inapparently carry pathogenic organisms on their penis or within their semen. Such bacteria as *Taylorella equigenitalis,* certain serotypes of *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* can produce an acute endometritis.

Taylorella equigenitalis is the causative organism of contagious equine metritis (CEM). *T. equigenitalis* is a gram-negative coccobacillus that is transmitted venereally and is highly contagious in equines. CEM was first documented 25 years ago in Newmarket, England. CEM has now been reported in horses worldwide. In July 1977, CEM was reported on 28 breeding farms in the Newmarket area, with approximately 200 Thoroughbred mares and 23 stallions affected.[26] By the conclusion of the 1978 breeding season, CEM was diagnosed in numerous countries, including the United States and Australia.[27] *T. equigenitalis* has now been isolated in 14 countries, infecting both Thoroughbreds and non-Thoroughbreds.[28]

In 1978, two strains of the organism were isolated from Kentucky Thoroughbreds.[29] One strain was sensitive to streptomycin and the other was resistant. In 1979, Missouri reported an outbreak of CEM in non-Thoroughbreds involving the streptomycin-sensitive strain.

Mares diagnosed with CEM reportedly exhibit shortened diestrous periods and copious mucopurulent vulvar discharges lasting up to 14 days. Stallions covering infected mares were asymptomatic. Numerous cultures were taken without isolating *T. equigenitalis* until fresh cultures were placed in transport medium and grown on chocolate blood agar incubated at 37°C in 5% to 10% carbon dioxide for 48 hours.[30] *T. equigenitalis* was found to be sensitive to a wide range of antibiotics and antiseptics, including benzyl penicillin, ampicillin, and chlorhexidine, but was resistant to streptomycin.[27]

A code of practice was issued in 1977 to control the spread of CEM. The code was published in the United Kingdom and similar measures were taken in Ireland and France. The code stipulated a mare as high risk if she cultured positive for CEM, was covered by an infected stallion, or if she arrived from outside the United Kingdom. The code mandated that high-risk mares should have a negative clitoral culture before and after arrival at stud and a negative endometrial and clitoral culture during estrus prior to breeding. Low-risk mares were required to have a negative clitoral swab taken before or after arrival at stud, providing there was agreement with the stud farm manager.[31] The code further stipulated that stallions should be cultured from pre-ejaculatory fluid, the penile sheath, the urethra, and the urethral fossa, and that all cultures should be sent to designated laboratories and incubated under microaerophilic and aerobic conditions. As a result of this code of practice, the incidence of CEM has decreased. Owing to strict importation regulations in the United States, CEM has not been reported in this country since 1983.[32]

Etiology

In 1978, the initial bacteriologic examination of swabs from the genitourinary tracts of infected animals revealed the CEM organism to be *Haemophilus equigenitalis* strain NCTC1/184 (61717/77). The organism was renamed *Taylorella equigenitalis* in 1984.[33]

Samples from animals suspected of CEM are shipped to the laboratory in Amies transport medium supplemented with charcoal. Upon arrival, specimens are transferred to chocolate blood agar, which is incubated in 5% to 10% carbon dioxide at 37°C. Pinpoint-sized, grayish colonies with a smooth outline appear by 48 hours. After 72 hours, the colonies can reach 1 to 2 mm in diameter and appear raised and shiny.

Transmission

Spread of CEM is primarily by venereal contact. Contaminated equipment, instruments, and personnel that contact the genital tracts of mares and stallions contribute to the spread of the disease. A carrier state exists because *T. equigenitalis* persists indefinitely in the clitoral sinuses of mares and urethral fossae of stallions. *T. equigenitalis* has been isolated from foals born to infected mares.[34] *T. equigenitalis* has been isolated also from an aborted fetus and placenta, but a repeated culture of the dam did not reveal the organism.[35]

Diagnosis

Diagnosis of CEM is confirmed by isolation of *T. equigenitalis* from the appropriate sites in the mare and stallion. It is imperative that samples be placed immediately in transport medium and shipped to a laboratory that is familiar with culture techniques for CEM.

Cultures should be taken during early estrus from the endometrium or cervix. Cultures from the clitoral fossa and the three clitoral sinuses should be submitted. The clitoral sinuses are small and can be visualized when the clitoris is partially extruded and held downward. A small swab moistened with sterile saline can be used to sample the sinuses. Clitoral cultures can be obtained at any time during the estrous cycle and from pregnant mares. A stallion's penis must be extended and samples taken from the

urethral fossa, urethra, preputial folds, skin of the penis, and pre-ejaculatory fluid.[28] Three negative cultures should be taken before the stallion is considered free of the disease.

Serology

Numerous serologic tests have been developed to detect antibodies to CEM in serum. The enzyme-linked immunosorbent assay (ELISA) and passive hemagglutination tests are superior for detection of infected mares.[28] Antibodies can be detected within 40 days of infection. The tests are useful in identifying mares with active infections. Carrier mares and infected stallions have no humoral response, and serologic tests are of little value as screening tests. *Taylorella asinigenitalis* and *B. ureolyticus* have shown such similarity in morphology and phenotype that there have been problems in the identification and conformation of *T. equigenitalis*.[36,37] The culture LightCycler PCR eliminates the confusion because it does not amplify *B. ureolyticus* DNA and discriminates from *T. asinigenitalis*.[38] Therefore, it can be used in conjunction with culture to fulfill export criteria and improve specificity and sensitivity.

Control

The code of practice instituted in 1977 for the control of CEM has dramatically reduced the incidence of the disease. Sporadic outbreaks have occurred that can be traced to carrier animals.[39] Carriers are considered high-risk animals and are treated and followed with extensive bacteriologic cultures until they are found to be negative. Continued vigilance against inapparent carrier animals has prompted countries, including the United States, to institute strict import regulations. Mares and stallions imported from countries not declared free of CEM must undergo quarantine and extensive testing at a designated quarantine station.[39]

Treatment

Intrauterine infusion of antibiotics combined with thorough cleansing of the clitoris, clitoral fossa, and clitoral sinuses is the treatment of choice in mares. Daily intrauterine infusions for 5 to 7 days with penicillin (5 to 10 million U), ampicillin, neomycin, and nitrofurazone have been reported to be successful.[28] The clitoral body, the fossa, and the sinuses must be thoroughly scrubbed daily for 5 days with 4% chlorhexidine solution and packed with nitrofurazone or chlorhexidine ointment. Clitoral sinusectomy might be recommended in mares that continue to harbor CEM in the clitoral area following treatment.

In stallions, the penis should be extended and then both the penis and prepuce thoroughly washed in a 4% chlorhexidine solution. The fossa glandis, urethral fossa, and prepuce are packed with nitrofurazone, penicillin, or chlorhexidine ointment. This should be repeated daily for 5 days. This treatment has been found to be highly successful.

ENDOMETRIOSIS

Endometriosis is a chronic degenerative condition of the endometrium.[40,41] It occurs in older mares that have been exposed to repeated inflammatory conditions or aging.[41] These severe changes can be a sequela of vesicovaginal reflux (urine pooling), chronic bacterial contamination, or delayed uterine clearance with inflammatory byproducts remaining in the uterine lumen. Unfortunately, the process has been found to be irreversible and untreatable and those mares that do get in foal have a harder time maintaining the pregnancy to term.

CHRONIC INFECTIOUS ENDOMETRITIS

Chronic endometritis is a major cause of equine infertility in older and multiparous mares. These mares become contaminated either during breeding or due to anatomic defects of the perineal and vulvar region leading to pneumovagina and fecal aspiration.[42] The most common organisms found in persistent endometritis are *Streptococcus zooepidemicus, Escherichia coli,* yeasts (*Candida* spp. and *Aspergillus* spp.), *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*.[43,44] Anaerobes have also been described as possibly playing a role.[45] In addition, the unique property of *S. zooepidemicus* enables it to physically adhere to the endometrium in susceptible mares with an increased appearance noted in mares with category 3 endometria.[46]

It is thought that in some mares with delayed uterine clearance, defective uterine immune defense mechanisms may contribute to persistence of infection.[47] Neutrophils need to migrate from the blood into the uterine lumen, phagocytose, and kill bacteria appropriately. Defects in the migration of blood-derived neutrophils have not been found in susceptible mares and factors in uterine secretions of susceptible mares have been determined to interfere with neutrophil phagocytosis rather than an innate phagocytic failure.[17,48] Opsonization by uterine secretion is dependent on both complement and specific antibody; and although a deficiency in complement was suggested to contribute to uterine defense failure, hemolytic complement was recognized to be elevated in flushings from susceptible mares.[49–52] Specific endometrial antibodies are important in the elimination of bacterial infection with their opsonic activity differing between susceptible and resistant mares.[53–55]

Cellular immunity has not received much attention in the equine uterus, although susceptible mares have normal macrophage function and no deficiency of T lymphocyte subsets.[15] A deficiency in antigen processing and handling at the uterine level may be due to a lack of an appropriate increased response of macrophages.[15]

Diagnosis of uterine inflammation due to persistent infection is based on history, physical examination, speculum examination, uterine cytology, uterine culture, and uterine biopsy. It is imperative that the practitioner use all available tests and not rely on only one.

Documentation of inflammation in the uterine lumen and accurate proof of an infectious organism is imperative.[56] In mares with infectious endometritis, the vaginal

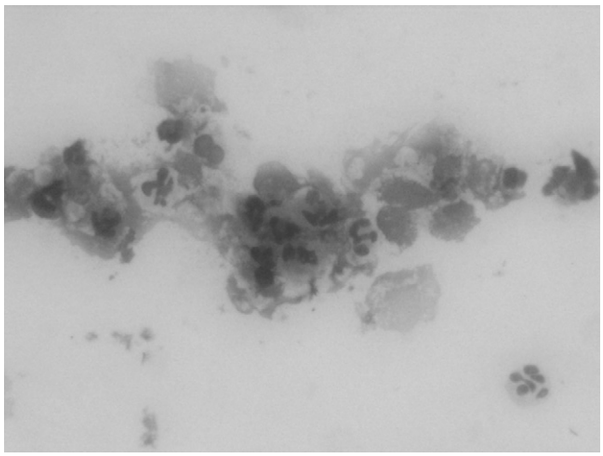

Fig. 20-1 Cytologic examination of endometrial cells with over 5 neutrophils per high-power field representing inflammation.

vault and cervix appear hyperemic on speculum examination with or without the presence of exudate. Rectal palpation may reveal a large relaxed uterus, and transrectal ultrasonography may show flocculent intraluminal fluid accumulation, the extent of which is dependent on the causative agent and severity. Endometrial cytologic examination is the diagnostic test of choice because it provides direct evidence of the presence of neutrophils in the uterine lumen. Any mare that has more than 5 neutrophils per high-powered field confirms active inflammation[56] (Fig. 20-1). Cytologic samples can be acquired by a guarded endometrial swab; collecting cells from the cap of a calajan culturette; or a small sterile guarded atraumatic brush or small volume uterine flush. Care must be taken to roll, not smear, the cells so as not to damage them. A representative sample contains both endometrial and inflammatory cells, with the best results obtained from centrifugation of a small volume uterine flush.[57] Blood contamination can produce a false positive cytologic finding. A guarded uterine culture confirms the causative organism and the antibiotic sensitivity patterns help to direct the treatment. The course of therapy undertaken will be determined by the cause of inflammation.

The goal of therapy for bacterial endometritis is to remove the offending bacteria and enhance uterine defense mechanisms, thereby decreasing the inflammatory process within the uterus. This has been accomplished with intrauterine infusions of antibiotics, antiseptics, and plasma; uterine lavage; ecbolics; and systemic antibiotic therapy. Therapy can be completed before or after breeding, depending on the severity of the infection. It is important to tailor the therapy to the individual case and to remember that no single treatment can be effective in all cases. With severe infections, multiple therapeutic approaches can be used to control the infection. This discussion attempts not to define a course of treatment but to illustrate successful and commonly used treatments. Every mare is an individual and the routine treatment of all mares with the same medication will not result in the best treatment.[58]

Intrauterine Therapy

A common form of therapy is intrauterine infusion of antibiotics, chemicals (antiseptics), and plasma.[59] Intrauterine infusion concentrates medication locally in the infected endometrium as opposed to systemic treatment, which relies on blood concentration of the drug.

For intrauterine antibiotic therapy, a drug should be selected on the basis of sensitivity tests. Broad-spectrum antibiotics might not be as effective as bactericidal antibiotics. Bacteriostatic drugs require assistance from the mare's defense system, which is likely compromised as indicated by the presence of infection.[59] Drug combinations should be avoided because incompatibility often renders the drugs ineffective. The same is true of drug and antiseptic combinations.[58]

The antibiotics most commonly used for intrauterine therapy are penicillin, gentamicin, ampicillin, nitrofurazone, polymyxin B, Timentin, ticarcillin, the sulfonamides, and amikacin.[58,60] Gentamicin, amikacin, ampicillin, and Timentin are the most effective against the majority of pathogenic organisms. Penicillin is thought to be one of the most effective antibiotics available for treating equine uterine infections.[58]

Antibiotics infused into the uterus are dissolved or suspended in sterile water or saline solution and infused directly into the uterus daily for 3 to 5 days or more during estrus.[56]

The size of the uterus, determined by transrectal palpation, should be used to determine the volume infused. The antibiotic can be suspended in volumes between 60 and 100 ml based on the assumption that this volume is optimal for coating the endometrial surface; however, the effects of such dilution on antibiotic efficacy are unknown. Present evidence suggests that most of the antibiotic introduced in this manner is expelled through the cervix soon after treatment.[56,59,61]

Intrauterine antibiotic therapy is not without risk. Indiscriminate use of antibiotics may alter the normal uterine flora and result in development of resistant strains of bacteria, yeast, and fungi.[58,62] Some mares are sensitive to particular antibiotics.[56] Caution must be used to ensure that therapy does not pose a greater threat than infection. The use of products not approved for intrauterine infusion can cause harmful effects in mares.[58]

Immunostimulants have been implicated in aiding the immune system to combat chronic endometritis. Studies are under way to try to objectively prove if they are in fact beneficial.

Uterine Lavage

For uterine lavage lactated Ringer or saline solutions are infused into the uterus via gravity and then allowed to drain. This should be repeated until the flush solution returning from the uterus is clear. A Bivona catheter with balloon and extension tubing can be connected directly

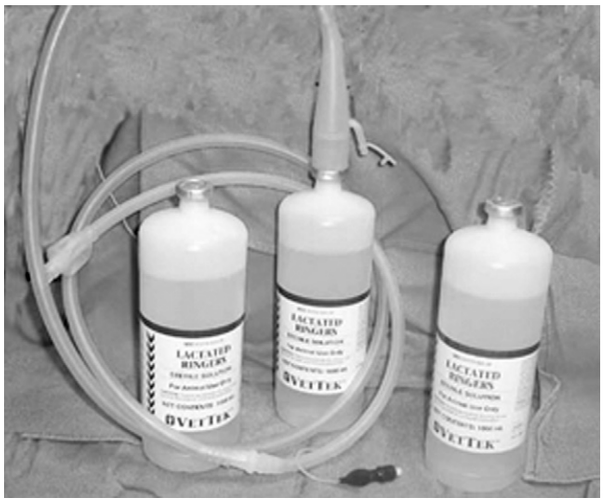

Fig. 20-2 Bivona balloon catheter with extension tubing to connect to lavage fluids.

to the fluid flush bottles (Fig. 20-2). Inspection of the recovered fluid provides immediate information concerning uterine health or the success of uterine treatments. The degree of cellularity and concentration of other inflammatory components correlates well with the appearance of the recovered fluid.[63] Note should be taken that older, multiparous mares have a larger uterus and therefore greater volumes are needed to be infused to fully distend and completely lavage the uterus (4–6 L of lactated Ringer solution). The rationale for this therapy is enhancement of cellular and mechanical aspects of uterine defense mechanisms.[63] The therapeutic effects of lavage are mechanical removal of bacteria and debris from the uterus; stimulation of uterine contractions, thus aiding expulsion of foreign material; and mild endometrial irritation resulting in migration of neutrophils and perhaps serum-derived opsonins to the uterine lumen.[64,56] Uterine lavage has also been reported to stimulate blood flow, improve tone, and decrease uterine size.[65] This procedure in combination with uterine ecbolics such as oxytocin or cloprostenol further enhances uterine clearance.[66] When the fluid is heated uterine lavage has proved beneficial in increasing myometrial tone and uterine circulation in older, multiparous mares with thick-walled or pendulous uteri.[56,63,65,67] Care must be taken, however, not to make the fluid too hot, causing endometrial scalding and adhesions.

Antiseptics

Antiseptics used historically for treatment of uterine disease included acriflavine, bismuth subnitrate, boric acid, charcoal, chlorine, iodine and iodine solutions, iodoform, perboric acid, silver oxide, hot saline and hypertonic saline, sodium hypochlorite, gentian violet, and hydrogen peroxide. Weak solutions of hydrogen peroxide have been used as treatment for acute endometritis and also appear helpful when exudate has been found in the uterine lumen.[59] After phagocytosis by neutrophils, bacteria are destroyed in part by oxidative metabolism, which includes hydrogen peroxide.[59] Lugol's solution (10%) has been reported to be successful for chemical curettage of the endometrium.[60,68]

Extreme care should be taken when infusing antiseptics not to inflict further damage with harsh or concentrated chemicals. A mare's uterus is sensitive to irritating substances.[56,60] Chlorhexidine suspension should be avoided because it causes tissue necrosis.[59,60,69] Oxytetracycline powder can also induce severe tissue necrosis. The endometrium can recover from some irritants, but repeated use may leave a fibrotic endometrium and possibly adhesions.[69] Additionally, hypertonic saline has been viewed by the author to cause severe endometrial adhesions on hysteroscopic examination.

Systemic Antibiotics

Antibiotics for treatment of uterine infections can be administered systemically. Opinions are divided on the success of systemic administration compared to intrauterine therapy. The majority appear to favor intrauterine infusion, especially for treatment of endometritis. Infused antibiotics are believed to be more effective because of direct contact of drugs with the infected endometrium. Systemic therapy has its supporters, and there are reports of success in treating endometritis systemically.[58,68,70] However, objective comparison between systemic and local antibiotic therapy is difficult without controlled trials.[70]

The main question to be addressed when considering antibiotic therapy is which tissues are involved. If the infection includes deeper layers of the uterus or other genital organs, then systemic therapy might be indicated. Some researchers believe that systemic administration results in higher antibiotic concentrations in infected tissues. Metritis, pyometra, and perimetritis would warrant systemic treatment.[58,59,68]

Systemic antibiotic therapy does have advantages. It can be conducted without invading the reproductive tract and treatment can be continued into the diestrous phase of the cycle, whereas postbreeding intrauterine therapy must stop before the embryo enters the uterus. Systemic therapy also enjoys an ease of application.[56,71] A disadvantage is the cost. Systemic therapy requires a higher dose to achieve effective blood concentrations.[56,69]

Based on satisfactory results reported by practitioners, antibiotics suitable for systemic treatment include amikacin sulfate, ampicillin, gentamicin, procaine penicillin, and trimethoprim sulfa.[56] Ciprofloxacin and probenecin has been used successfully by the author and co-workers to combat difficult cases of endometritis due to *Pseudomonas* spp.

Plasma Therapy

Plasma or colostrum augmentation of a mare's natural defense mechanism is another avenue of uterine disease treatment. Plasma therapy is often indicated when no causative agent is identified, when sensitivity tests indicate impractical drugs, or when previous therapy has been unsatisfactory.[56] The goal of plasma therapy is to

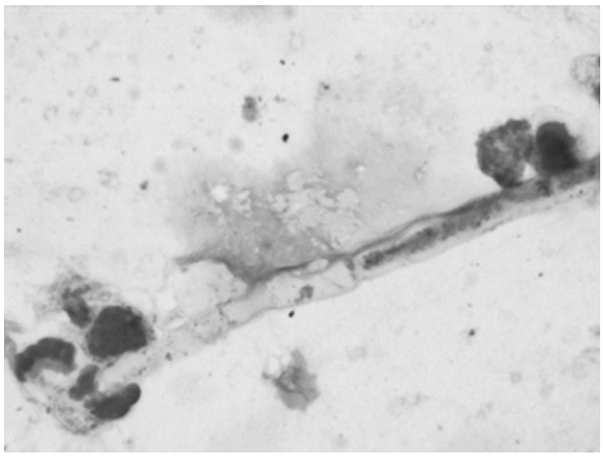

Fig. 20-3 Fungal hyphae with neutrophils.

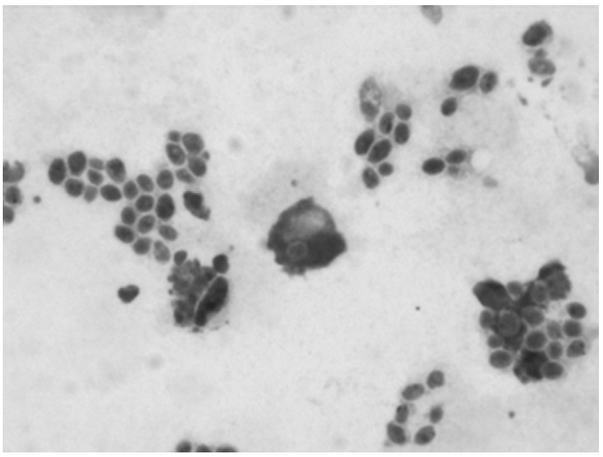

Fig. 20-4 Extra- and intracellular yeasts.

enhance the local uterine immune response and natural defense mechanism. Failure or incompetence of these responses probably accounts for mares that are unable to cope with contaminating organisms.[65] Since plasma is the major source of proteins involved in opsonization of bacteria in the uterus, it has been suggested that a practical method to overcome ineffective uterine defense mechanisms is to infuse plasma or colostrum, substances that contain antibodies, into the uterine lumen.[56,65]

Studies have utilized both homologous and heterologous plasma. The use of heterologous plasma would be convenient, but the possibility of uterine sensitization to foreign protein cannot be ruled out.[67]

Antibiotic and plasma therapy can be used in concert, but some antibiotics are more compatible with phagocytic processes than others. Studies have found that in concentrations commonly used for intrauterine infusion, amikacin sulfate and gentamicin sulfate inhibit neutrophil phagocytosis, and potassium penicillin and ticarcillin do not.[56,67] Further work is needed in this area.

The topic of chronic infectious endometritis would not be complete without the mention of fungal endometritis. The treatment of endometritis due to fungus or yeast is far more difficult than bacteria. *Candida* spp. and *Aspergillus* spp. are the most common fungal organisms associated with fungal endometritis. Poor conformation allowing contamination of the reproductive tract from the environment, repeated or lengthy intrauterine antibiotic treatment, and excessive reproductive manipulation have all been implicated as potential causes of fungal invasion of the uterus.[72] Diagnosis can be made most successfully by endometrial cytologic examination revealing branching fungal hyphae or budding yeast in the presence of inflammatory cells (Figs. 20-3 and 20-4). Endometrial cultures plated on blood or Sabouraud's agar can also aid in identification. Ultrasonography can reveal large amounts of intraluminal flocculent fluid. Endometrial biopsy is recommended in recurrent infections to ascertain the extent of endometrial invasion and inflammation. A white purulent vaginal discharge may also be noted. Treatment has been attempted using uterine lavage to decrease fungal numbers in conjunction with DMSO, vinegar (2%), or dilute Betadine (0.05%). Antifungal agents such as clotrimazole (500–700 mg), amphotericin B (100–200 mg), fluconazole (100 mg) and nystatin ($0.5–2.4 \times 10^6$) can also be used.[44]

Unfortunately studies to determine the necessary length of treatment for a successful outcome have not been done, and therefore, therapy failure may be due to inadequate treatment duration due to cost inhibition. New research has advocated the use of Lufenuron (540 mg) in fungal endometritis.[73]

Lufenuron is a benzoylphenyl urea derivative and is classified as an insect development inhibitor that prevents the synthesis, polymerization, and deposition of chitin.[74] Fungal organisms are surrounded by chitin-rich cell walls, and it is therefore speculated that disruption of this cell wall inhibits fungal growth.[73] In a practical situation the jury is still out concerning whether or not Lufenuron adequately alleviates fungal endometritis. Correcting underlying conformational defects is advised to prevent recurrent infection. Prognosis for reproductive performance is unfortunately poor, due to endometrial invasion of organism and attachment in endometrial folds; treatment only affects certain stages of fungal development, reinfection, delayed uterine clearance, and inadequate duration of treatment. At this time, early diagnosis and treatment is our best defense.

PERSISTENT MATING-INDUCED ENDOMETRITIS

A normal inflammatory response occurs after mating in which there is an influx of PMNs into the uterine lumen due to a chemotactic effect of spermatozoa.[11] It has been speculated that this chemotactic role of spermatozoa may be necessary to clear the uterus from excess spermatozoa and seminal plasma.[10,12] However, this inflammatory process is normally delayed due to the suppression of phagocytosis and migration of PMNs by seminal plasma, therefore allowing spermatozoa to reach the oviduct for fertilization to take place.[75] Seminal plasma has therefore

been suggested to modulate the inflammatory response to spermatozoa, explaining why mares inseminated with frozen/thawed semen containing minimal seminal plasma develop marked and prolonged postbreeding endometritis.[12] During the activation of PMNs, $PGF_{2\alpha}$ is released, causing myometrial contractions.[76] These myometrial contractions are regulated by $PGF_{2\alpha}$ with oxytocin.[11] Uterine contractions are needed to remove contaminants and inflammatory products, thereby preventing persistent inflammation and endometrial damage. If there is a sustained inflammatory process after ovulation, luteolysis will occur and the uterine environment will not be conducive for embryo development. This can cause a substantial reduction in fertility. Susceptibility to persistent mating-induced endometritis (PMIE) is defined as the inability of the estrous mare to clear intraluminal fluid accumulation within 12 to 48 hours after breeding.[16,77] The primary mechanism responsible for the continued presence of intraluminal fluid is impaired physical clearance due to reduced myoelectrical activity and motility.[23,78] Not only has evacuation of the uterus after mating been shown to be defective in mares with PMIE, but sperm transport to the oviduct is also affected.[15] In addition, volume of inseminate may also influence persistence of uterine inflammation, and a recent study has shown that larger volumes decrease the inflammatory response.[79] Intraluminal fluid, which in most cases is indicative of inflammation when seen after mating, can be detected by transrectal ultrasonography.[80,81] The incidence of intrauterine fluid 1 to 2 days after natural breeding has been reported to be between 15% and 43% in the general mare population, with pregnancy rates being significantly lower in these mares.[24,82]

Diagnosis of PMIE should be considered when a mare retains intrauterine fluid for more than 12 hours after breeding.[83] This fluid can be revealed with transrectal ultrasonography in the absence of bacterial contamination. However, mares that are suspected of having this problem should have a complete history obtained, a complete evaluation prior to breeding to include vulvar and perineal anatomy, normal cervical relaxation and closure, cytologic testing and culture, transrectal ultrasonography, and a uterine biopsy. Severe histopathologic changes in the uterine biopsy correlate well with susceptibility to PMIE.[17]

Treatment is aimed at assisting the uterus to physically clear contaminants and inflammatory products. This can be done using large volume uterine lavage 6 to 12 hours after mating in susceptible mares in conjunction with oxytocin 3 to 12 hours after mating.[84,85] $PGF_{2\alpha}$ has also been suggested to help with uterine clearance, with increased myoelectrical activity occurring for 5 hours after 10 mg $PGF_{2\alpha}$ while 20 units of oxytocin causes only 1 hour of increased activity.[83,86] Further studies have revealed that dose and interval between treatments has an effect on efficacy with peak intrauterine pressures occurring in the first 5 to 10 minutes after oxytocin administration and a reduced effect on contractile activity in subsequent treatments if given at intervals of 2.4 hours.[87,88] Decreased pregnancy rates have been shown when treatment consisted of 25 units compared with 15 units of oxytocin after breeding.[89] Therefore, it is the recommendation of the authors to combine uterine lavage in combination with oxytocin or cloprostenol within 6 to 12 hours after breeding. Practically speaking, however, this may not be possible, in which case oxytocin or cloprostenol can be used followed by uterine lavage when the mare is checked the following day. Oxytocin may be used two to three times daily with the authors preferentially using cloprostenol at night when the mares are stabled with less activity. Care must be taken not to use cloprostenol after ovulation because some evidence suggests that it may have a transient effect on corpus luteum formation and progesterone production. Additionally, acupuncture in conjunction with the preceding therapies has been shown to be successful in reducing intraluminal fluid and increase pregnancy rates in mares with PMIE.[90] Regimens used by the author incorporate treatment initiated at the onset of estrus with a treatment delivered every other day until 2 days after ovulation.

METRITIS

Metritis is an inflammation or infection of the deeper layers of the uterus. Systemic signs of metritis are not seen with any degree of regularity but occur most frequently in postpartum mares and mares that have a retained placenta. Draft mares seem to be particularly susceptible to septic metritis. Septic metritis may extend to the peritoneal surface of the uterus (perimetritis) and into the peritoneal cavity (parametritis). Clinical signs of septic metritis include depression, increased temperature (>39.4° C), loss of appetite, and laminitis. Septic metritis can be life-threatening and requires prompt treatment. Large volume uterine lavage with broad-spectrum systemic antibiotics, anti-inflammatories, and preventives for laminitis are imperative for successful results.

Pyometra is characterized by accumulation of purulent exudate in the lumen of the uterus with a closed cervix. The uterus is distended as in pregnancy, and the cervix usually has adhesions due to previous trauma or surgery that prevents drainage. Progesterone concentrations are elevated as a result of a retained corpus luteum, and the endometrium is unable to release prostaglandin F_2 alpha ($PGF_{2\alpha}$). Mares do not show systemic signs. The majority of pyometra cases are diagnosed during routine examination. Treatment can be discouraging because of the permanent damage that may have occurred to the endometrium as well as the inability to resolve cervical adhesions or deformation. Prognosis can be aided by uterine biopsy.

Prevention of uterine disease necessarily precedes any consideration of uterine disease treatment. The importance of a sound prevention and management program cannot be overstated. This is crucial even for mares with normal uterine defense responses because continual contamination debilitates the uterus. Older, multiparous mares and postpartum mares recovering from foaling difficulties have ineffective or compromised uterine defense responses. These mares are particularly susceptible to infection. A sound prevention program begins with sanitary breeding, foaling, and examination procedures. Care must be taken to minimize contamination. Mares prone to infection from organisms introduced at breeding

should be bred near the time of ovulation to reduce unnecessary breedings.[56,58,60] These mares often warrant postbreeding uterine treatment. Surgical correction of anatomic defects also precedes uterine therapy, because abnormal conformation permits bacterial contamination. Caslick's operation corrects poor vulvar conformation that results in pneumovagina.[58–60,67] Third-degree perineal lacerations, rectovaginal fistulas, cervical lacerations, and urine pooling can be corrected with surgery, thus improving fertility.[56,58,59,61]

SALPINGITIS

Salpingitis, or inflammation of the oviduct, is a lesion of principal importance because minor inflammatory changes are incompatible with successful passage of spermatozoa to the ovum and journey of the embryo to the uterus. Conception is rendered impossible (1) by occlusion of the lumen through acute or proliferative swelling, (2) by the lethal effect of toxic inflammatory exudates upon spermatozoa, and (3) by destruction of stretches of ciliated epithelium or contractile muscle that propels the ovum to the uterus.[91] The incidence of salpingitis, however, is low due to the disposition of sphincter muscle around the uterine ostium.[82] This tight uterotubal junction appears to prevent the ascent of endometritis. The special anatomic position of the uterus of the mare in which the oviducts are in the dorsal part of the abdominal cavity above the uterus, rather than lying ventrally, also aids in the prevention of inflammation.[93] Salpingitis in the mare appears to be a widespread infiltrative, nonocclusive, and generally nonexudative process.[94] Past studies, of which there are few, revealed that there is a much higher incidence of ampullitis than isthmitis, as well as twice as great a chance for the two conditions to be bilateral.[93]

In 80% of cases, isthmitis has been associated with endometritis.[93] This could be due to the fact that the highly folded mucous membrane of the ampulla is more sensitive and an inflamed isthmus could recover more easily and rapidly than the ampulla.[93] Infundibulitis may be explained by the presence of the larval form of *Strongylus edentatus,* which migrates over serosal surfaces. Follicular fluid and blood released at ovulation may contribute to the formation of infundibular adhesions. The blood clots could become organized into adhesions, which could then become secondarily infected by hematogenously derived organisms.[93] A hypersensitivity reaction in the infundibulum has also been suggested.[93] Unfortunately, evidence is still lacking concerning how salpingitis, whatever the origin, affects fertility. Additionally, inflammation of the oviduct does not seem to be synonymous with oviductal blockage, and therefore, care should be taken to diagnose infertility due to salpingitis until further evidence and tests can be utilized to determine significance.[93] Flushing of the oviducts appears to be a solution to some, when no other cause of infertility is found; however, care must be taken because this procedure is not without consequences, and adhesions can be produced at the uterotubal junction, causing occlusion and further problems. Therefore, this procedure should be used judiciously.

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CHAPTER 21

Bacterial Causes of Subfertility and Abortion in the Mare

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Bacterial infections of the uterus are the leading cause of subfertility and infertility in mares and represent a major economic loss to the equine industry.[1] The equine breeding industry depends on the health of the uterus, and without a healthy uterus a mare cannot successfully conceive or carry a foal to term. The industry's demand for early foaling dates is largely responsible for equine infertility. The mare's natural breeding season is late May, June, July, and August. In the early spring mares are in reproductive transition and therefore estrus periods are irregular and prolonged. Ovulation is irregular and difficult to predict, and repeated examinations are required to detect it. More than one breeding is usually necessary, which results in added contamination of both the mare and stallion. Frequent uterine contamination and disease occur if a breeder does not implement a meticulous preventive management program to minimize uterine contamination. This program must include immediate recognition and treatment of infected mares.

Bacteria enter the uterus at foaling, breeding, during routine genital examinations, and through vulvar defects (pneumovagina). The uterus of a healthy mare is able to respond to contamination and evacuate transient bacterial contamination. If the uterus is contaminated at breeding, normal defense mechanisms eliminate bacteria without treatment and re-establish a normal environment before the embryo descends into the uterus. Older, multiparous mares and postpartum mares often require special attention and management because their natural defenses have been compromised, and uterine contamination in these susceptible mares often results in infection.[2–6]

CLINICAL SIGNS

Endometritis, which is inflammation of the endometrium, can occur with or without bacterial infection and can be acute or chronic. Most young mares normally resolve uterine contamination within 96 hours, and these mares are considered to be resistant to endometritis.[7] Mares unable to clear bacterial contaminants from their uterus become susceptible, and this results in persistent inflammation. Susceptible mares may have other conformational defects, such as pneumovagina, urine pooling, and foaling injuries, that predispose them to endometritis.

Metritis is an inflammation or infection of the deeper layers of the uterus. Systemic signs of metritis are not seen with any degree of regularity but occur more frequently in postpartum mares. Draft mares seem to be particularly susceptible to septic metritis. Septic metritis may extend to the peritoneal surface of the uterus (perimetritis) and into the peritoneal cavity (parametritis). Clinical signs of septic metritis include depression, increased temperature (>39.4°C), loss of appetite, and laminitis. Septic metritis can be life-threatening and requires prompt treatment.

Pyometra is characterized by accumulation of purulent exudate in the lumen of the uterus with a closed cervix. The uterus is distended and the cervix usually has adhesions that prevent drainage. Progesterone concentrations are elevated as a result of a retained corpus luteum, and the uterus is unable to release prostaglandin F_2 alpha ($PGF_{2\alpha}$). Mares do not show systemic signs. The majority of pyometra cases are diagnosed during routine examination.

PATHOGENESIS

Many different bacteria have been isolated from mares with uterine disease, but *Streptococcus zooepidemicus* followed by *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* are the most frequently isolated pathogens.[8–10] *Corynebacterium* spp., *Proteus* spp., and *Staphylococcus* spp. are considered pathogenic when isolated from mares with cytologic evidence of uterine disease. *Gardnerella vaginalis,* an organism reported to cause vaginitis in humans, has been documented in mares[11,12]; however, biopsy samples indicated only mild endometritis, and the mares conceived and carried foals to term without antibiotic therapy, so the clinical significance is questionable.[12] *Taylorella equigenitalis* is a pathologic, microaerophilic, gram-negative organism transmitted venereally and is the cause of contagious equine metritis (CEM). *Bacteroides fragilis* is an anaerobic organism that has been associated with acute endometritis at foal heat and in barren mares.[13]

DIAGNOSIS

Diagnosis of uterine disease is based on history, physical examination, vaginal examination, uterine cytologic examination, uterine culture, and uterine biopsy. It is imperative that the practitioner use all available tests and not rely on only one. Diagnosis of infectious endometritis is based on documentation of inflammation in the uterine lumen and accurate proof of an infectious

organism.[14] Endometritis may escape detection with vaginal examination, transrectal uterine palpation, and uterine ultrasonography. In these cases, the uterus is not enlarged and there is only a small amount of accumulated fluid or exudate. Endometrial cytologic examination is the diagnostic test of choice because it provides direct evidence of the presence of neutrophils in the uterine lumen. Any significant number of neutrophils confirms active inflammation.[14] A uterine culture confirms the causative organism and the antibiotic sensitivity helps direct the treatment. The Accu-CulShure (ACCU-MED Corp., Pleasantville, NY) swab is an efficient tool for obtaining endometrial specimens. When culturing the endometrium, it is important to obtain a sample uncontaminated by the vestibule and vagina caudal to the urethral orifice because this area is usually heavily contaminated with normal flora. The Accu-CulShure swab is sealed inside the guard tube and resealed immediately following sample collection, so there is no contamination or exposure to air during entry or egress. When the instrument has been removed from the genital tract following specimen collection, the swab is slowly pulled into the self-contained transport medium. The protected swab can be snapped off at the score on the instrument, placed in a transport tube, and sent to a diagnostic laboratory. Metritis and pyometra are associated with uterine enlargement and fluid (purulent exudate) accumulation in the uterus. The fluid may extend to the cervical or vaginal area. Therefore, vaginoscopy, transrectal uterine palpation, and uterine ultrasonography clearly aid in diagnosis. A uterine culture identifies the causative organism, and a biopsy identifies the extent of the disease.

THERAPY

The goal of therapy is to remove or reduce the offending bacteria and enhance uterine defense mechanisms, thereby decreasing the inflammatory process within the uterus. This has been accomplished with intrauterine infusions of antibiotics, antiseptics, plasma, sugars, uterine lavage, and systemic antibiotic therapy. Therapy can be completed before or after breeding, depending on the severity of the infection. It is important to tailor the therapy to the individual case and to remember that no single treatment can be effective in all cases. With severe infections, multiple therapeutic approaches can be used to control the infection. This discussion attempts not to define a course of treatment but to illustrate successful and commonly used treatments. Every mare is an individual and the routine treatment of all mares with the same medication will not result in the best treatment.[15]

Intrauterine Therapy

A common form of therapy is intrauterine infusion of antibiotics, chemicals (antiseptics), plasma,[16] and sugars.[17,18] Medications are infused by gravity directly into the uterus, thus contacting the entire surface of the endometrium. Intrauterine infusion concentrates medication locally in the infected endometrium as opposed to systemic treatment, which relies on blood concentration of the drug.

For intrauterine antibiotic therapy, a drug should be selected on the basis of sensitivity tests. Broad-spectrum antibiotics might not be as effective as bactericidal antibiotics. Bacteriostatic drugs require assistance from the mare's defense system, which is likely compromised as indicated by the presence of infection.[16] Drug combinations should be avoided because incompatibility often renders the drugs ineffective. The same is true of drug and antiseptic combinations.[15] The antibiotics most commonly used for intrauterine therapy are penicillin, gentamicin, ampicillin, streptomycin, chloramphenicol, nitrofurazone, polymyxin B, neomycin, the sulfonamides, and amikacin.[15,19] Gentamicin, amikacin, ampicillin, and chloramphenicol are the most effective against the majority of pathogenic organisms. Penicillin is thought to be one of the most effective antibiotics available for treating equine uterine infections.[15]

Antibiotics infused into the uterus are dissolved or suspended in sterile water or saline solution and infused directly into the uterus daily for 3 to 5 days or more during estrus.[20] Antibiotic treatment after service is advised for mares with impaired uterine resistance that cannot cope with microorganisms introduced at breeding. Antibiotics can be instilled into the uterus up to 3 days after ovulation, but treatment may be wasted if the mares require a second breeding.[21] The size of the uterus, determined by transrectal palpation, should be used to determine the volume infused. The uterine capacity of maiden mares is approximately 35 ml, and that of older mares may be between 60 and 150 ml.[16] Traditionally, the antibiotic is suspended in large volumes of fluid (100–500 ml) on the assumption that this volume is optimal for filling the uterus and coating the endometrial surface; however, the effects of such dilution on antibiotic efficacy are unknown. Present evidence suggests that most of the antibiotic introduced in this manner is expelled through the cervix soon after treatment.[16,20,21] Intrauterine antibiotic therapy is not without risk. Indiscriminate use of antibiotics may alter the normal uterine flora and result in development of resistant strains of bacteria, yeasts, and fungi.[5,15] Some mares are sensitive to particular antibiotics.[20] Caution must be used to ensure that therapy does not pose a greater threat than infection. The use of products not approved for intrauterine infusion can cause harmful effects in mares.[15]

Uterine Lavage

For uterine lavage, large volumes of saline solution or sterile water are infused into the uterus and then allowed to drain. The rationale for this therapy is enhancement of cellular and mechanical aspects of uterine defense mechanisms.[2] The therapeutic effects of lavage are mechanical removal of bacteria and debris from the uterus; stimulation of uterine contractions, thus aiding expulsion of foreign material; and mild endometrial irritation resulting in migration of neutrophils and perhaps serum-derived opsonins to the uterine lumen.[4,20] Uterine lavage has also been reported to stimulate blood flow, improve tone, and decrease uterine size.[6] When the fluid

is heated to 40° to 50° C, lavage has proved beneficial in increasing myometrial tone and uterine circulation in older, multiparous mares with thick-walled or pendulous uteri.[2,6,20,22] Lavage solutions are infused into the uterus in 1-L increments either by gravity or mechanically and then siphoned into a clear, graduated container.[20] Inspection of the recovered fluid provides immediate information concerning uterine health or the success of uterine treatments. The degree of cellularity and concentration of other inflammatory components correlates well with the appearance of the recovered fluid.[2]

Antiseptics

Antiseptics used historically for treatment of uterine disease included acriflavine, bismuth subnitrate, boric acid, charcoal, chlorine, iodine and iodine solutions, iodoform, perboric acid, silver oxide, hot saline and hypertonic saline, sodium hypochlorite, gentian violet, and hydrogen peroxide. Weak solutions of hydrogen peroxide have been used as treatment for acute endometritis and also appear helpful when exudate has been found in the uterine lumen.[16] After phagocytosis by neutrophils, bacteria are destroyed in part by oxidative metabolism, which includes hydrogen peroxide.[22] Lugol's solution (10%) has been reported to be successful for chemical curettage of the endometrium.[6,23]

Care should be taken when infusing antiseptics not to inflict further damage with harsh or concentrated chemicals. A mare's uterus is sensitive to irritating substances.[6,18] Chlorhexidine suspension should be avoided or used cautiously because it causes tissue necrosis.[6,16,24] Oxytetracycline powder can also induce severe tissue necrosis. The endometrium recovers well from some irritants, but repeated use may leave a fibrotic endometrium and possibly adhesions.[24]

Systemic Antibiotics

Antibiotics for treatment of uterine infections can be administered systemically. Opinions are divided on the success of systemic administration compared to intrauterine therapy. The majority appear to favor intrauterine infusion, especially for treatment of endometritis. Infused antibiotics are believed to be more effective because of direct contact of drugs with the infected endometrium. Systemic therapy has its supporters, and there are reports of success in treating endometritis systemically.[8,15,23] However, objective comparison between systemic and local antibiotic therapy is difficult without controlled trials.[8] The main question to be addressed when considering antibiotic therapy is which tissues are involved. If the infection includes deeper layers of the uterus or other genital organs, then systemic therapy might be indicated. Some researchers believe that systemic administration results in higher antibiotic concentrations in infected tissues. Metritis, pyometra, and perimetritis may warrant systemic treatment.[15,16,23]

Systemic antibiotic therapy does have advantages. It can be conducted without invading the reproductive tract and treatment can be continued into the diestrous phase of the cycle, whereas postbreeding intrauterine therapy must stop before the embryo enters the uterus. Systemic therapy also enjoys an ease of application.[20,25] A disadvantage is the cost. Systemic therapy requires a higher dose to achieve effective blood concentrations.[20,24] Based on satisfactory results reported by practitioners, antibiotics suitable for systemic treatment include amikacin sulfate, ampicillin, gentamicin, procaine penicillin, and trimethoprim sulfa.[20]

Plasma Therapy

Plasma or colostrum augmentation of a mare's natural defense mechanism is another avenue of uterine disease treatment. Plasma therapy is often indicated when no causative agent is identified, when sensitivity tests indicate impractical drugs, or when previous therapy has been unsatisfactory.[20] The goal of plasma therapy is to enhance the local uterine immune response and natural defense mechanism. Failure or incompetence of these responses probably accounts for mares that are unable to cope with contaminating organisms.[6] These natural mechanisms are defined as cellular (phagocytes) and noncellular (opsonins, thermal stabile factors, and the leukocytic tide).[6] The uterus of resistant mares contains opsonins, which function to allow efficient phagocytosis of contaminating bacteria by neutrophils. It is believed that susceptible mares are deficient in opsonins, which allows bacteria to become established.[8] Plasma is the major source of proteins involved in opsonization of bacteria in the uterus.[20] It has been suggested that a practical method to overcome ineffective uterine defense mechanisms is to infuse plasma or colostrum, substances that contain antibodies, into the uterine lumen.[6]

Studies have utilized both homologous and heterologous plasma. The use of heterologous plasma would be convenient, but the possibility of uterine sensitization to foreign protein cannot be ruled out.[22] Antibiotic and plasma therapy can be used in concert, but some antibiotics are more compatible with phagocytic processes than others. Studies have found that in concentrations commonly used for intrauterine infusion, amikacin sulfate and gentamicin sulfate inhibit neutrophil phagocytosis, and potassium penicillin and ticarcillin do not.[14,22] More work is needed in this area.

Intrauterine Sugar Solutions

Because of an emergence of bacteria becoming resistant to multiple antibiotics, it. will be imperative to seek alternatives to antibiotic therapy in the future. Monosaccharide sugars have been shown to be effective in inhibiting bacteria that commonly infect the genitourinary tract of mares. One of the benefits of sugars is that they can be used alone or with antibiotics to treat uterine infections in mares. Specific sugars can inhibit bacterial adherence to the equine endometrium in vitro. Mannose and *N*-acetyl-D-galactosamine inhibited adhesion of *Escherichia coli* and *Pseudomonas aeruginosa* to epithelial cells, whereas only mannose inhibited adhesion of *Streptococcus zooepidemicus*. In horses with uterine infections, use of sugars to competitively displace bacteria from attach-

ment sites on cells may provide an adjunct to antibiotic treatment.[17]

Bacterial adherence to cell surfaces and phagocytosis of bacteria have become important in relation to pathogenicity of various strains of bacteria that are common causes of uterine infections, including *Escherichia coli, Pseudomonas aeruginosa, Streptococcus zooepidemicus, Salmonella* spp., and other bacteria. The adherence of bacteria is inhibited by sugars such as L-fructose and D-galactose, which suggest that sugar-mediated adherence is widespread. The intercellular recognition is thought to be mediated by sugar residues such as D-mannose on the surface of cells to which bacteria attach by sugar-binding substance of their surface. The nature of the receptors is unknown, but there is evidence that bacteria such as *E. coli* produce lectin-like substances specific for D-mannose, by which it binds to the cells.[18]

Management and Prevention

Prevention of uterine disease necessarily precedes any consideration of uterine disease treatment. The importance of a sound prevention and management program cannot be overstated. This is crucial even for mares with normal uterine defense responses because continual contamination debilitates the uterus. Older, multiparous mares and postpartum mares recovering from foaling difficulties have ineffective or compromised uterine defense responses. These mares are particularly susceptible to infection. A sound prevention program begins with sanitary breeding, foaling, and examination procedures. Care must be taken to minimize contamination. Mares prone to infection from organisms introduced at breeding should be bred near the time of ovulation to reduce unnecessary breedings.[15,19,20] These mares often warrant postbreeding uterine treatment. Surgical correction of anatomic defects also precedes uterine therapy, because abnormal conformation permits bacterial contamination. A Caslick operation corrects poor vulvar conformation that results in pneumovagina.[15,16,19,22] Third-degree perineal lacerations, rectovaginal fistulae, cervical lacerations, and urine pooling can be corrected with surgery, thus improving fertility.[15,16,20,21]

CONTAGIOUS EQUINE METRITIS

Taylorella equigenitalis is the causative organism of contagious equine metritis (CEM). *T. equigenitalis* is a gram-negative coccobacillus that is transmitted venereally and is highly contagious in equines. CEM was first documented almost 30 years ago in Newmarket, England. CEM has now been reported in horses worldwide.

History

In July 1977, CEM was reported on 28 breeding farms in the Newmarket area, with approximately 200 Thoroughbred mares and 23 stallions affected.[26] By the conclusion of the 1978 breeding season, CEM was diagnosed in numerous countries, including the United States[29] and Australia.[27] *T. equigenitalis* has now been isolated in 14 countries, infecting both Thoroughbreds and non-Thoroughbreds.[28]

In 1978, two strains of the organism were isolated from Kentucky Thoroughbreds.[29] One strain was sensitive to streptomycin and the other was resistant. In 1979, Missouri reported an outbreak of CEM in non-Thoroughbreds involving the streptomycin-sensitive strain. Mares diagnosed with CEM reportedly exhibit shortened diestrous periods and copious mucopurulent vulvar discharges lasting up to 14 days. Stallions covering infected mares were asymptomatic. Numerous cultures were taken without isolating *T. equigenitalis* until fresh cultures were placed in transport medium and grown on chocolate blood agar incubated at 37°C in 5% to 10% carbon dioxide for 48 hours.[30] *T. equigenitalis* was found to be sensitive to a wide range of antibiotics and antiseptics, including benzyl penicillin, ampicillin, and chlorhexidine, but resistant to streptomycin.[27]

A code of practice was issued in 1977 to control the spread of CEM. The code was published in the United Kingdom and similar measures were taken in Ireland and France. The code stipulated a mare as high risk if she cultured positive for CEM, was covered by an infected stallion, or if she arrived from outside the United Kingdom. The code mandated that high-risk mares should have a negative clitoral culture before and after arrival at stud and a negative endometrial and clitoral culture during estrus prior to breeding. Low-risk mares were required to have a negative clitoral swab taken before or after arrival at stud providing there was agreement with the stud farm manager.[31] The code further stipulated that stallions should be cultured from pre-ejaculatory fluid, the penile sheath, the urethra, and the urethral fossa, and that all cultures should be sent to designated laboratories and incubated under microaerophilic and aerobic conditions. As a result of this code of practice, the incidence of CEM has decreased. [32] However, recently three bacterial isolates of *Taylorella asinigenitalis* sp. *nov.* were isolated from the urethral fossa from one male donkey in California and from two male donkeys in Kentucky; these isolates were identical to each other, but different from *Taylorella equigenitalis*. The isolates were phenotypically indistinguishable from *Taylorella equigenitalis,* but sequence analysis of DNA encoding the 16S rRNA revealed that the gene sequences of these isolates were virtually identical to each other (>99.8% similarity), but different (97.6% similarity) from those of several confirmed isolates of *T. equigenitalis*. Based on these findings, a new different species named *Taylorella asinigenitalis* sp. *nov.* was proposed.

Mares bred to infected donkeys did not show clinical signs of disease, even though infection with the organism was established in those bred naturally, but did produce antibodies that reacted in the complement fixation test utilized to identify mares recently infected with *T. equigenitalis*.[33]

Etiology

In 1978, the initial bacteriologic examination of swabs from the genitourinary tracts of infected animals revealed the CEM organism to be *Haemophilus equigenitalis* strain

NCTC1/184 (61717/77). The organism was renamed *Taylorella equigenitalis* in 1984.[34]

Samples from animals suspected of CEM are shipped to the laboratory in Amies transport medium supplemented with charcoal. Upon arrival, specimens are transferred to chocolate blood agar, which is incubated in 5% to 10% carbon dioxide at 37°C. Pinpoint-sized, grayish colonies with a smooth outline appear by 48 hours. After 72 hours, the colonies can reach 1 to 2 mm in diameter and appear raised and shiny.

Transmission

Spread of CEM is primarily by venereal contact. Contaminated equipment, instruments, and personnel that contact the genital tracts of mares and stallions contribute to the spread of the disease. A carrier state exists because *T. equigenitalis* persists indefinitely in the clitoral sinus of mares and urethral fossa of stallions. *T. equigenitalis* has been isolated from foals born to infected mares.[35] *T. equigenitalis* has been isolated also from an aborted fetus and placenta, but a repeated culture of the dam did not reveal the organism.[36]

Diagnosis

Diagnosis of CEM is confirmed by isolation of *T. equigenitalis* from the appropriate sites in the mare and stallion. It is imperative that samples be placed immediately in transport medium and shipped to a laboratory that is familiar with culture techniques for CEM.

Cultures should be taken during early estrus from the endometrium or cervix. Cultures from the clitoral fossa and the three clitoral sinuses should be submitted. The clitoral sinuses are small and can be visualized when the clitoris is partially extruded and held downward. A small swab moistened with sterile saline can be used to sample the sinuses. Clitoral cultures can be obtained at any time during the estrous cycle and from pregnant mares. A stallion's penis must be extended and samples taken from the urethral fossa, urethra, preputial folds, skin of the penis, and pre-ejaculatory fluid.[28] Three negative cultures should be taken before the stallion is considered free of the disease.

Recent molecular advances utilizing the polymerase chain reaction (PCR) amplification and nucleotide sequencing of the 16S ribosomal DNA sequence have been confirmed to be effective for identification of *T. equigenitalis*. These new analytical methods at the genomic DNA level also enabled the discrimination of the newly discovered donkey-related *T. asinigenitalis* from *T. equigenitalis*.[37] A culture-LightCycler PCR assay has been developed for the detection of *T. equigenitalis,* the causative agent of CEM in horses. The culture-LightCycler PCR assay is specific, sensitive, and reproducible, and can be used effectively for the detection of *T. equigenitalis* infections and can also detect *T. asinigenitalis* isolates.[38]

Serology

Numerous serologic tests have been developed to detect antibodies to CEM in serum. The enzyme-linked immunosorbent assay (ELISA) and passive hemagglutination tests are superior for detection of infected mares.[28] Antibodies can be detected within 40 days of infection. The tests are useful in identifying mares with active infections. Carrier mares and infected stallions have no humoral response and serologic tests are of little value as screening tests.

Control

The code of practice instituted in 1977 for the control of CEM has dramatically reduced the incidence of the disease. Sporadic outbreaks have occurred that can be traced to carrier animals.[28] Carriers are considered high-risk animals and are treated and followed with extensive bacteriologic cultures until they are found to be negative. Continued vigilance against inapparent carrier animals has prompted countries, including the United States, to institute strict import regulations. Mares and stallions imported from countries not declared free of CEM must undergo quarantine and extensive testing at a designated quarantine station.[39]

Treatment

Intrauterine infusion of antibiotics combined with thorough cleansing of the clitoris, clitoral fossa, and clitoral sinuses is the treatment of choice in mares. Daily intrauterine infusions for 5 to 7 days with penicillin (5 to 10 million U), ampicillin, neomycin, and nitrofurazone have been reported to be successful.[28] The clitoral body, the fossa, and the sinuses must be thoroughly scrubbed daily for 5 days with 4% chlorhexidine solution and packed with nitrofurazone or chlorhexidine ointment. Clitoral sinusectomy might be recommended in mares that continue to harbor CEM in the clitoral area following treatment. In stallions, the penis should be extended and then both the penis and prepuce thoroughly washed in a 4% chlorhexidine solution. The fossa glandis, urethral fossa, and prepuce are packed with nitrofurazone, penicillin, or chlorhexidine ointment. This should be repeated daily for 5 days. This treatment has been found to be highly successful.

BACTERIAL ABORTIONS

The rate of abortion in mares is between 5% and 15%. Abortions that occur early in gestation, between conception and 90 days, often go undetected and are frequently confused with infertility. The incidence of abortion between 20 and 90 days ranges from 7% to 30%.[40–43] Most observed abortions occur after the fourth and fifth months of gestation. The incidence of abortions after this period is approximately 2% to 12% of all pregnancies.[44] The causal agents involved in equine abortions at all stages of gestation have been divided into infectious, noninfectious, and unknown.[45,46] Infectious causes can account for as many as 47.5% to as few as 16.2% of abortions.[40] Bacteria are the major cause of abortions in mares.

Bacterial Causes of Abortions

Bacteria can cause abortion at any stage of gestation.[42,47] Bacteria associated with equine abortion are: *Streptococcus zooepidemicus; Streptococcus equisimilis* and other streptococci; *Escherichia coli; Pseudomonas aeruginosa; Staphylococcus aureus* and other staphylococci; *Klebsiella pneumoniae* var *genitalium; Leptospira* spp.; nocardioform actinomycetes; and *Salmonella abortus equi*.[43–46,48] The highest incidence of bacterial abortions occurs between the fifth and tenth months of gestation. Bacteria have been implicated in early abortions, but this theory is based on the isolation of organisms from uterine cultures following abortion.[49] Bacteria cannot be implicated merely by their presence; there must be evidence of autolysis and inflammatory changes determined by examination of fetal tissue and the placenta.[43] Organisms enter the fetoplacental unit through the maternal circulation as a result of systemic infection; by ascent through the cervix and infection of the placenta and fetus[44,47]; or possibly, although there is no conclusive evidence, from deep-seated endometritis.[50] The majority of clinicians and researchers agree that the cervix is the most common route.[40,44,47,50]

Streptococcus zooepidemicus is a common cause of abortion at any gestational stage. *S. zooepidemicus* is found on the external genitalia of mares and stallions and is the most common organism associated with vaginitis, cervicitis, and metritis. *S. zooepidemicus* usually ascends through the cervix and causes placentitis.[44] Streptococcal placentitis is a frequent cause of fetal abortion. Gestation continues until the infection compromises the placenta and the pregnancy can no longer be maintained. Abortion occurs after fetal distress or death. The infection can also spread to the fetus and cause septicemia, death, and abortion.[44,50] Streptococcal infections occur most frequently on poorly managed farms that utilize unsanitary breeding, foaling, and examination procedures; excessive breeding of older mares; and frequent services at foal heat.[44] *E. coli, P. aeruginosa, S. aureus,* and *K. pneumoniae* can also ascend through the cervix owing to pneumovagina and result in placentitis associated with progressive placental insufficiency and abortion.[50] Nocardioform actinomycetes are gram-positive, non–acid fast, filamentous branching bacteria.[48] The bacteria do not belong to any of the four recognized species of *Nocardia*. Recent studies have further classified one of these nocardioform actinomycetes, which appears to cause the majority of cases of nocardioform placentitis, as *Crossiella equi,*[51] and similar actinomycete strains have also been further classified.[52] These bacteria cause characteristic placentitis that results in late-term abortions, stillbirths, and premature birth of near-term foals.[48] The characteristic lesions are distributed at the base of the gravid and nongravid horns or at the junction between the body and horn of the placenta. The affected areas are thickened, and the chorionic surface is covered with brown, sticky, mudlike material and dotted with white granular structures. The exposed chorionic surface is red and white, mottled, and roughened. These nocardioform actinomycetes induce extensive chronic placentitis, which grossly mimics mycotic placentitis.[48,53] Mares that abort a fetus with these nocardioform organisms seem to clear of infection fairly rapidly without therapy.

The prevention of bacterial abortions caused by agents ascending through the cervix begins with sanitary breeding and examination procedures, pre- and postbreeding uterine therapy when necessary, and careful management of problem mares (Caslick's procedure).

Salmonella abortus equi was infamous during the late 1800s as the cause of contagious equine abortions, but except for two small isolated outbreaks, *Salmonella* abortions have not been reported in the United States since 1932.[44] The organism is transmitted by ingestion of feed and water contaminated by feces or genital discharges.

Leptospira spp. have also been identified with increasing frequency as a cause of abortion, stillbirth, and premature live birth.[54] Abortion results from fetal infection. The highest incidence of leptospiral abortion occurs during the seventh through eleventh months.[40,48,54] The serotypes commonly associated with equine abortions are *pomona, grippotyphosa,* and *bratislava*.[54–56] *Leptospira* infect the fetus hematogenously, usually after a mild 1- to 3-week maternal illness. Clinical signs include elevated body temperature, anorexia, jaundice, and slight depression.[44,56,57] *Leptospira* have zoonotic potential and are spread by accidental infection. Flu-like symptoms, meningitis, and hepatorenal failure have been reported in infected humans.[56]

Most horses have antibodies against a wide range of serotypes with *pomona, grippotyphosa,* and *bratislava* being the most common. In the horse, serotype *bratislava* is considered to be host-adapted, and *pomona* and *grippotyphosa* are non–host-adapted.[56] Horses infected with a host-adapted serotype can serve as reservoir hosts (persistent shedding in urine), whereas a non–host-adaptive serotype is transmitted as an accidental or incidental disease. Host factors, not the organism itself, determine how long the serotype can be maintained.[56] Mild clinical signs are associated with host-adapted serotypes, and more severe signs are associated with non–host-adapted serotypes. Most abortions are the result of non–host-adapted serotypes *(pomona)* in mares.[56] Clinical signs are rarely observed but include pyrexia, icterus, anorexia, and depression.[58] Abortions occur 1 to 3 weeks after the clinical signs. Abortions occur at any time but most often after the sixth month of gestation. A high percentage of abortions occur in November and December, an observation that has been attributed to wet climatic conditions, horse management conditions, contamination from feral animals, or a combination of these factors.[59] An aborted fetus can display no gross lesions, but it may have a swollen yellow liver, perirenal edema, or white radiating streaks in the kidney, pneumonia, and placentitis. The placenta can have gross lesions, which include slimy exudate, cystic allantoic glands, and a thickened allantochorion. Histologically, the placental lesions consist of focal necrosis of villi, mild perivasculitis, cystic allantoic glands, and infiltration of neutrophils and mononuclear cells in the stroma and villi. Spirochetes can be confirmed by Warthin-Starry stain from the kidney or placenta.[55]

Leptospiral antibodies can be detected in the aborted fetus and in maternal blood. Fetal antibodies are detected

in body fluids or blood or both. Titers range from 1:50 to greater than 1:638,400. Affected mares have antibody titers greater than or equal to those found in the fetus (1:200 to 1:3,276,800).[55] A second voided urine sample (following intravenous administration of furosemide) collected from mares with a two- to fourfold increase in antibody titer should be examined by culture and fluorescent antibody tests to demonstrate leptospires.[60]

Transmission is thought to occur by contact with sources of environmental infection. Animals should be fenced away from low-lying or swampy areas, stagnant water, and runoff water from other animals (cattle, pigs, dogs, or wildlife).[55] Horses can shed the organism for up to 3 months (2 to 3 weeks is normal) and should be isolated (D. Williams, personal communication, 1994).[56] Control of the disease consists of isolation of shedder animals and urine testing until three negative fluorescent antibody tests are completed (D. Williams, personal communication, 1994). A *Leptospira* vaccine approved for use in mares does not exist. Vaccination of horses with cattle vaccine produces extremely high titers (>1:50,000), but these vaccines are not currently approved and do not contain all the appropriate serotypes.[56,60] Vaccines may also pose a threat of anaphylactic reactions in horses.[60] Mares shedding the organism can be treated with procaine penicillin G (20,000 IU/kg IM twice a day) or oxytetracycline (5 mg/kg, IV twice a day) to decrease the shedding period.[58] One trial found that antibiotics administered for 5 days did not eliminate shedding of *Leptospira* in the urine. Administration of antibiotics for longer periods of time may be effective. Antibiotics are thought to be useful in preventing fetal infections in mares with high titers during late pregnancy, thus facilitating the delivery of healthy foals.[56] Leptospiral abortions occur during the wet months and usually affect only one or two mares in a herd. It is important to prevent contamination by isolating infected animals and instituting strict sanitation measures. Contact with infected urine, aborted fetuses, or contaminated feed and water must be eliminated. Stalls, water buckets, and feed troughs should be thoroughly disinfected.

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CHAPTER 22
Equine Herpesvirus Infections

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Herpesviral infection is most commonly a mild disease of the upper respiratory tract of young and adult horses caused by three related herpesviruses that are widespread throughout the world. Equine herpesvirus 1 (EHV-1) is most commonly diagnosed as a cause of abortion during late pregnancy.

ETIOLOGY

Equine herpesviruses are classified as EHV-1 to EHV-5.[1–3]

1. EHV-1 is the most important cause of abortion as well as the cause of respiratory disease, neonatal death, and neurologic disease in the mare.
2. EHV-2 (equine cytomegalovirus) causes mild long-term respiratory infection in foals.[4]
3. EHV-3 causes coital exanthema.
4. EHV-4 causes respiratory disease throughout the year, and sporadic abortion (rhinopneumonitis).
5. EHV-5 has unknown pathogenicity.[5]

EPIDEMIOLOGY

Since the first report of equine herpesviral abortion in 1932,[6] EHV-1 and EHV-4 have been widespread in the United States, Canada, Japan, Australia, and Europe. Although horses of all ages are infected, signs of upper respiratory disease are usually observed in young horses. The viruses are highly infectious, and infection originates directly or indirectly from other horses. Transmission occurs by inhalation of infected droplets or by ingestion of food or water contaminated by nasal discharges or aborted fetuses. EHV-1 can survive for 14 to 42 days outside horses.[7] Up to 80% of horses in the United States have serologic evidence of exposure to EHV-1 infection. Cattle and donkeys have been reported to be possible reservoirs of the virus, and infection by EHV-1 has been reported in llamas, alpacas, deer, antelope, and gazelle.[8]

Carriers are necessary for EHV-1 to persist from year to year. Being a typical herpesvirus, infection may persist in a latent state and be reactivated subsequently by various stressors, for example, corticosteroids.[9] As immunity to EHV-1 infection is short and transient, horses may be clinically infected a number of times. Cell-mediated immunity is important in the resistance to herpesviruses.

The EHV-1 causes inflammation of the respiratory tract characterized by coughing and nasal discharge. The initial viral infection is most severe, secondary viral infections are less severe, and subsequent infections tend to produce few or no clinical signs. Fatalities in uncomplicated cases are unlikely.

Most outbreaks of abortion are associated with outbreaks of respiratory disease in foals and yearlings several weeks earlier, commonly in the fall and winter. Separation of weanlings and yearlings from pregnant mares is important in the control of EHV-1 abortion. "Storms" of abortions may occur in bands of broodmares, with up to 90% at risk of either aborting or losing their foals during the first day of life. Most herpesviral abortions occur sporadically in a herd, with maiden mares most often affected. Multiple abortions may occur in closed herds or in open herds with additions or transients from other farms or racetracks.[10] Multiple abortions are most common on farms where pregnant mares are crowded and where there are additions during the final months of the foaling season. Such conditions produce stress and favor epidemic spread of EHV-1 virus. The prevalence of abortion is higher in mares transported during late pregnancy and in those that have gone through sales. Multiple abortions in all age groups usually indicate a lack of immunity in a closed herd that was recently exposed to the EHV-1 virus by a new addition. Abortion is rare in mares kept in a group with no new mares added after the first trimester of pregnancy.[10]

As the majority of breeding mares are clinically immune to the respiratory but not to the abortigenic infection, spread of EHV-1 infection occurs commonly in a herd without obvious clinical signs. Although pregnant mares abort 60 days or more after intranasal exposure, most abort within 30 days. Spread of infection within a group of pregnant mares exposed to a mare that aborted from EHV-1 infection depends on the status of their immunity to respiratory tract infection. The most dangerous source of infection to pregnant mares is an infected aborted fetus. Aborting mares do not shed virus from either the respiratory or reproductive tract at the time of abortion.[10]

Neurologic signs associated with abortion were observed in the 1940s, but isolation of EHV-1 from nervous tissue was not recorded until 1966. It has been reported with increasing frequency since.[7]

PATHOGENESIS

Infection with EHV-1 is responsible for at least four distinct syndromes in horses: respiratory, abortigenic, neonatal death, and neurologic.[2,7,10,11] EHV-1 is highly contagious, and transmission is by inhalation of infectious aerosols or by direct contact with infectious secretions or contaminated drinking water.[6,11] EHV-1 replicates in the epithelial cells of the nasal, pharyngeal, and tonsillar mucosae and then spreads to the regional lymph

nodes. That stage is followed by leukocyte-associated viremia and infection of vascular endothelium in a number of organs.[6,11,12] From that point, invasion of lungs, endometrium, chorioallantois, fetus, and nervous tissue may occur.[11–15] It is surmised that EHV is acquired as a respiratory disease leading to latency and that abortion occurs as an unpredictable sequela to reactivation in a carrier mare.[16]

Spread of EHV-1 occurs transplacentally to fetuses by infected leukocytes, and abortion occurs within 120 days, with the majority occurring in 7 to 20 days.[6,11,15] The longer period may result from persistence of EHV-1 in leukocytes in the endometrium or chorioallantois before it invades the fetus and causes death and abortion. The virus damages the endometrium and the chorioallantois. Local edema occurs at the fetomaternal junction, leading to separation of the chorioallantois from the endometrium and the fetus dies from anoxia; near-term fetuses may be expelled alive but are nonviable. The fetus is usually infected and lesions include massive lymphocytic destruction in the thymus and spleen. Experimental studies have revealed that extensive damage to the endometrium from widespread vasculitis, thrombosis, and ischemia, with replication of EHV-1 in endothelial cells, may occur without evidence of infection in aborted fetuses.[15] EHV-1 has been isolated from fetuses aborted by mares with high levels of serum-neutralizing antibodies to EHV-1.[17]

Abortions usually occur between 7 months of gestation and term, with no maternal illness evident at that time.[10] Respiratory disease before abortion may not be observed in mares, as EHV-1 infection may have been mild, subclinical, or latent; latent disease may have been reactivated by stress-induced immunosuppression.[6,9,11,16–18]

Prenatal infection with EHV-1 without concurrent abortion or respiratory disease has been reported from Australia and resulted in stillbirths or weak, nonviable foals that died within a few hours to 24 hours of birth, and foals apparently normal at birth that developed severe respiratory distress within 18 to 24 hours and died within 24 to 72 hours.[19–21] A different prenatal EHV-1 infection resulting in neonatal death in older foals was reported from Kentucky and was characterized by respiratory disease, diarrhea, and weakness during the first weeks of life.[22] The foals were normal at birth, were agammaglobulinemic, and survived for a few weeks before succumbing to a variety of secondary bacterial and viral infections.

The pathogenesis of the neurologic form of EHV-1 infection is complex and uncertain. The relationship to reinfection and the microscopic changes in the central nervous system suggest an immune-mediated vasculitis typical of a type III hypersensitivity reaction.[11,13,14] Infection is considered to spread to the central nervous system (CNS) through EHV-1-infected leukocytes. Outbreaks of EHV-1 myeloencephalitis appear to be caused by certain strains of EHV-1.[13,16] Originally thought to be confined to pregnant mares, the EHV-1 paralytic syndrome has been observed in barren mares, stallions, geldings, and foals.[23–25] Experimentally, only pregnant mares in early or midpregnancy, not the last trimester, could be infected.[11,13,14,25] The outcome of EHV-1 infection of the spinal cord and brain varied from recovery to permanent locomotor impairment or death.

CLINICAL FINDINGS

Respiratory Disease

Severity of EHV-1 respiratory disease is age-related, with acute upper respiratory tract infection occurring mainly in foals, weanlings, and yearlings. After an incubation period of 2 to 20 days, signs include fever (39° to 40.5°C), conjunctivitis, leukopenia (largely neutropenic[26]), serous to mucopurulent nasal discharge, and cough. The lymph nodes of the throat may be slightly enlarged. The course of illness is usually 2 to 5 days, but the nasal discharge and cough may persist for 1 to 3 weeks.[6,7,11,18,26] Inapparent EHV-1 infections are common. Infection in older horses previously infected is mild to subclinical with few or no clinical signs. Secondary bacterial infection may result in pneumonia. Young horses may develop viral pneumonia.

Abortion

Prevalence of EHV-1 abortion in a group of mares varies from 1% to 90%, depending on management, degree of immunity, virulence of the virus, various stress factors, and number of exposed susceptible mares in advanced pregnancy. Although abortion storms may occur, most herpesviral abortions today tend to be sporadic. Abortions occur over a period of 2 weeks to 3 months, with the majority occurring within 60 days. Mares may abort up to 4 months after respiratory infection, which may be mild and unobserved. The incubation period following natural infection is usually 15 to 30 days. The incidence of abortion is highest in the last trimester, particularly at 8 to 10 months, but may occur as early as 5 months.

Mares usually abort suddenly without any premonitory signs, and the aborted fetus is normally fresh and usually attached to the fetal membranes. Some infected foals are born dead at term, and others may be liveborn and nonviable.[6,10–12,17] Aborting mares recover rapidly and their fertility is not compromised.[27]

Neonatal Mortality

Some infected foals are apparently normal at birth but become weak and depressed and die within 3 days with severe respiratory distress.[19,20] Other infected foals may survive for a few weeks before succumbing to a variety of secondary bacterial or viral infections.[22] They may have respiratory distress, diarrhea, and weakness during that period.

Neurologic Disease

Although EHV-1 myeloencephalitis is usually associated with respiratory disease and abortion,[23,26] it has been reported separately.[7] Outbreaks may involve up to 90% of mares, foals, stallions, and geldings on a breeding

farm.[23,25] Neurologic disease is more common in horses being reinfected with EHV-1. Modified live-vaccine virus may be involved, as nervous signs often occur soon after vaccination or natural or experimental infection.[18] The incubation period for both natural and experimental infection is about 7 days.[14,23,24]

Clinical signs vary from slight ataxia with recovery to severe ataxia followed by paresis that may last for weeks and is followed by recumbency leading to death or euthanasia. Recumbent horses are usually euthanatized within a month. Some horses die acutely after an illness of only 2 days. Urinary incontinence may also occur.[14,23–25]

LESIONS

Abortion

Fetal lesions in EHV-1 abortion vary greatly and depend on the stage of gestation. Before 6 months' gestation, the fetuses are autolytic with diffuse scattering of intranuclear inclusions without local inflammatory response. Later fetuses are usually aborted fresh with their fetal membranes. Fetuses affected at 7 to 10 months of gestation have widespread lesions in liver, lungs, thymus, adrenals, and lymph nodes, and toward term, the lesions are more severe in the lungs.[6,10] In general, the gross fetal lesions include mild icterus, subcutaneous edema, scattered petechiae, hydrothorax and hydroperitoneum, pulmonary edema, heavy and firm lungs, numerous, small, grayish white necrotic foci (1 to 2 mm) in the liver, and focal areas of necrosis in thymus, adrenals, spleen, and lymph nodes. Rarely are all these gross lesions found in the same fetus; the most consistent are pulmonary edema and hydrothorax and hepatic necrotic foci. Microscopically, the lesions are characteristic and diagnostic: viral eosinophilic intranuclear inclusions in necrotic foci in the liver and also in lung, thymus, adrenals, and lymph nodes. Necrosis of lymphocytes occurs in the thymus, the spleen, and the lymph nodes.[6,10,12] There is bronchiolitis and focal pneumonitis in aborted fetuses, and a diffuse pneumonitis in term foals.[10] These lesions do not resemble those found in any other cause of equine abortion.

Neonatal Mortality

Gross lesions in neonates dying within 3 days of birth include voluminous, firm lungs, often "plum purple" with massive atelectasis, submucosal hemorrhagic striations in the trachea and bronchi, and bronchial lymph nodes that are often enlarged, congested, and edematous. Microscopic changes include extensive nonsuppurative alveolitis, mild to severe acute focal necrotizing bronchitis, and bronchiolitis with eosinophilic intranuclear inclusions. Inclusions are frequently observed in the thymus but rarely in the liver. Focal adrenocortical necrosis and depletion or degeneration of thymic and splenic lymphocytes were observed in fewer than 50% of the affected foals.[21]

Neonates surviving for 2 weeks before succumbing had destructive lesions in splenic lymphocytes and massive necrosis of the thymus.[22] EHV-1 intranuclear inclusions were not observed.

Neurologic Disease

Neurologic effects include acute disseminated myeloencephalitis with widespread vasculitis of small blood vessels in the spinal cord and brain and thrombosis that results in hypoxic degeneration and hemorrhage in adjacent tissues and foci of malacia. Microscopic lesions are prominent in both natural and experimental EHV-1 myeloencephalitis.[13,14,25] Vascular changes are generally more prominent in the spinal cord than in the brain, and spinal cord lesions are more prominent in the white matter than in the gray matter.[14] Hemorrhages in the spinal cord and brain are associated with disseminated vasculitis as evidenced by extensive endothelial immunofluorescence and thrombosis within 4 days following experimental infection.[13]

Respiratory Disease

Equine herpesviral respiratory disease is rarely fatal and deaths result from secondary bacterial infections, especially streptococcal. Young horses may develop bronchopneumonia. The main lesion is rhinitis with extensive necrosis of the epithelial cells.[7,12]

DIAGNOSIS

Signs of upper respiratory tract infection in young and adult horses and late abortion with characteristic lesions in aborted fetuses are highly suggestive of EHV-1 infection.[6,10,11,16] Diagnosis can be confirmed by serology, virus isolation, and histopathology. Bacteriologic examination should be negative. Equine herpesviral infection must be differentiated from equine viral arteritis, equine influenza, and strangles. All equine abortions should be considered to be caused by EHV-1 until proved otherwise.

Serologic tests for detecting EHV-1 and EHV-4 include immunofluorescence, serum virus neutralization, enzyme-linked immunosorbent assay, polymerase chain reaction, and indirect immunoperoxidase.[28,29] Respiratory infection can be confirmed by isolation of the virus from nasal swabs submitted in virus transport medium or from blood samples. Polymerase chain reaction is a sensitive and rapid technique for detecting EHV-1 and EHV-4 in nasopharyngeal swabs.[28] A rising titer in virus-neutralizing antibodies in acute and convalescent blood samples is necessary to confirm a recent EHV-1 or EHV-4 infection.[8] A single positive sample confirms previous infection, as herpesvirus-neutralizing antibodies persist for over a year.

Abortion caused by EHV-1 can be confirmed by immunofluorescence, virus isolation, or histopathologic examination. Although histopathologic lesions are frequently pathognomonic, it is prudent to combine histopathologic testing with virus isolation and immunofluorescence.[25] Fluorescent antibody tests have been found to be highly specific and correlate closely with virus isolation and histopathologic findings.[25,30,31] Samples of fetal lung, liver, thymus, and adrenals should

be submitted frozen for virus isolation and immunofluorescence and fixed in 10% buffered neutral formalin for histopathologic examination. Immunostaining for EHV-1 can be performed on formalin-fixed paraffin-embedded sections. Rapid and effective diagnosis of EHV-1 can be provided by an indirect immunoperoxidase technique applied to frozen and paraffin-embedded sections of fetal liver.[29] Fetal and neonatal serologic findings may assist in the diagnosis of EHV-1 abortion, as serum from aborted fetuses, stillborn foals, and weak neonatal foals before colostrum ingestion may have significant virus-neutralizing antibody titers.[32] EHV-1 can be isolated from the brain and spinal cord of affected mares but not consistently.[8]

CONTROL

Sound management practices in conjunction with hygienic procedures to prevent spread of disease and a vaccination program offer the best means for prevention and control of EHV-1 infection.[27] Early recognition of an aborted fetus or a sick newborn foal is important in preventing spread of EHV-1. Aborted fetuses present the biggest risk to susceptible pregnant mares in the transmission of this highly contagious and infectious disease. Sick foals should be prevented from having contact with pregnant mares, the surroundings should be thoroughly disinfected, and all bedding should be burnt. Any aborted fetus and its membranes should be removed in a plastic bag or container for disposal, preferably to a diagnostic laboratory. If this is not possible, then a necropsy should be performed in a suitable, safe location that can be easily cleaned and disinfected. Samples should be collected, packed safely, and sent to a laboratory for diagnosis.

Aborting mares should be isolated and the contaminated area cleaned and disinfected. Attendants must take precautions not to infect other pregnant mares by carrying virus on their clothing, equipment, and utensils. That will require disinfecting boots and changing clothing before attending to other pregnant mares. Mares and foals should be observed closely for signs of abortion and illness. When abortion occurs, no mares should be allowed to enter or leave a breeding farm until EHV-1 infection is eliminated from the diagnosis.[6,11,27]

Management practices such as crowding of pregnant mares, visiting broodmares, indiscriminate mingling of horses of various age groups, the environment of sales, and other factors that produce stress can significantly influence the occurrence of EHV-1 disease.[8,27] Pregnant mares on a breeding farm with EHV-1 infection can leave, provided that they have been strictly isolated from aborted mares for a month after the last abortion. They should be kept isolated from pregnant mares at other breeding farms for an additional 2 months. Pregnant mares returning from an infected breeding farm to their home farm must be kept in isolation until they have foaled.

Vaccines are currently used in conjunction with good management to prevent outbreaks of EHV-1 respiratory disease, abortion, and neurologic disease. The principal objective with broodmares is to protect against abortion. Vaccines currently available are modified-live and inactivated EHV-1 vaccines, but the resulting immunity is of short duration.[6,7,27] The vaccines should contain EHV-1 and EHV-4, although some cross-protection occurs. Two sequential injections of EHV-1 vaccine protect against challenge with EHV-4, but two injections of EHV-4 vaccine do not protect against EHV-1.[33] The limited effectiveness of available vaccines is demonstrated by the continual outbreaks of herpesvirus abortion, respiratory disease, and neurologic disease despite intense vaccination programs.[8] New and more effective vaccines such as a living subunit vaccine and a thymidine kinase–negative EHV-1 deletion mutant vaccine are being tested and may become available in the near future.[7,27]

Cell culture–adapted modified live-virus vaccines are widely used on breeding farms. They do not spread among horses or cause respiratory disease or abortion. Frequent vaccinations are necessary to maintain immunity.[7,27] Broodmares should be vaccinated twice during the latter half of pregnancy, or at the fifth, seventh, and ninth months of gestation to maintain immunity during the period of maximum risk for abortion. Foals and yearlings should also be vaccinated if they are running with the mares or are on the same farms at the critical time of pregnancy when EHV-1 infection is likely to occur. Passive immunity wanes around weaning and this is when foals contract EHV-1 infection. It is recommended that foals should be vaccinated 2 weeks before weaning, followed by two subsequent injections at 4- to 6-week intervals, and a booster injection every 3 months. The level of protection against fetal and foal deaths is low, and other forms of protection such as management must be provided.[4]

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CHAPTER 23

Equine Viral Arteritis

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Equine viral arteritis (EVA) is an acute systemic to subclinical respiratory viral disease of horses characterized by generalized vascular necrosis and by abortion.[1–3] EVA usually occurs as a mild and often unrecognized infection of the anterior respiratory tract.[4] Initially a disease of Standardbreds, it now also affects Thoroughbreds.[5,6] It is apparently worldwide in distribution.

ETIOLOGY

The causal agent is an *arterivirus,* an RNA virus of the family Togaviridae. It is the sole member of the genus.[5] Several strains of the EVA virus with varying degrees of virulence have been reported, with only one antigenic type.[6–8] The prototype is the Bucyrus strain from the originally diagnosed outbreak,[1] and its virulence contrasts sharply with the clinically inapparent infections transmitted by many long-term carrier stallions.[6,9]

EPIDEMIOLOGY

Equine viral arteritis was first recognized in 1953 following an outbreak of severe respiratory disease and abortion in Bucyrus, Ohio, and additional outbreaks occurred in Ohio, Indiana, California, and Kentucky.[1] From then to 1984 there were relatively few reports of EVA, although infection appears to be widespread.[5,10,11] Outbreaks of clinical disease are infrequent and occur mainly in mares on breeding farms where up to 50% abort.

A serologic survey revealed 70% to 90% of Standardbred mares and less than 3% of adult Thoroughbreds to be EVA seropositive.[10] The first recorded outbreaks at racetracks in Standardbreds were reported in 1977. The outbreak of EVA in Kentucky in 1984 was the first reported occurrence in Thoroughbreds in North America.[5] There was considerable variation in severity in the 1984 outbreak, with affected mares exhibiting a variety of clinical signs from commonly reported EVA lesions to inapparent infection.[5,6] There were no associated deaths or confirmed cases of abortions. All affected mares made complete, uneventful recoveries. In confirmed outbreaks of EVA since 1984, abortion occurred infrequently.[6]

Horses are the only host of EVA virus, and all ages are susceptible. EVA spreads rapidly in groups of susceptible horses. Mortality rate is low in natural outbreaks, and after recovery, affected horses have prolonged immunity. Currently, the EVA virus is so avirulent that clinical signs are not obvious or are mild when sporadic outbreaks occur.[2,6,10]

TRANSMISSION

Susceptible mares can be infected by inhalation of aerosols from infected horses, and respiratory spread is a major method of transmission of EVA virus in barns, at pasture or sales, and at racetracks.[1,11] Droplets from nasal exudate of infected horses remain infectious for up to 10 days in the environment. Abortion from natural or experimental infection occurs 23 to 57 days after exposure and from 6 to 29 days after the onset of fever.[12]

Venereal transmission from infected stallions to susceptible mares played a significant role in the widespread dissemination of EVA during the 1984 outbreak.[6,9,13] Sexually infected mares transmitted EVA virus to susceptible mares by contact.[6,12] Virtually 100% transmission of EVA virus occurred in susceptible mares bred to long-term shedding stallions during the 1984 outbreak, so clinical infection must have been mild and undetected. Venereal transmission was a major method of spread of EVA on breeding farms.[5,6]

The role of indirect contact with virus-contaminated fomites varied with outbreaks.[6] The role of teaser stallions and nurse mares, although a potential risk, was apparently insignificant in the 1984 outbreaks.[6]

The EVA virus is present in fluids and tissues of aborted fetuses and placentae, which could potentially result in indirect lateral spread of infection.[1] Foals of immune mares are resistant to infection until the loss of colostral antibodies at 2 to 6 months of age.

Carriers of EVA virus are important in perpetuating infection in horse populations. The duration of the carrier state in stallions shedding the virus in semen can vary from weeks to years.[6,9] The frequency of long-term carriers is approximately 30% to 35%. Following experimental infection with EVA, the virus is shed in semen for months, and the ampulla and bulbourethral glands appear to be preferred sites for viral infection.[13] There is little evidence, however, to indicate that mares are EVA carriers with any appreciable frequency. Evidence of congenital infection in foals is lacking.[6]

PATHOGENESIS

The virus of EVA is pathogenic to endothelial cells and causes panvasculitis. In clinical EVA, the inhaled virus replicates initially in lung macrophages and spreads to the local lymph nodes. Viremia is followed by replication in endothelial and medial cells of small arteries and severe vascular damage, especially in the intestinal tract, visceral lymph nodes, and adrenals. That results in

increased permeability, generalized edema, and hemorrhage. Pulmonary edema and hydrothorax are manifested by dyspnea. Hemorrhagic enteritis results in diarrhea and abdominal pain. Petechiae of mucosae and conjunctivae, and edema of limbs also occur. Arterial damage peaks at day 10 after infection when edema has largely disappeared. The vascular lesions resolve if the horse survives. In severe cases, death may occur from dehydration, hypotension, and severe hypoxia.[2,3,14]

Abortion apparently results from severe necrotizing myometritis, placental detachment, decreased progesterone, and local prostaglandin release.[14] Aborted fetuses may be alive or dead but rarely have lesions attributable to EVA. Virus may be isolated from the placenta and fetal spleen and lung.[14]

CLINICAL SIGNS

Infections associated with EVA may vary from acute to mild to subclinical; most are subclinical with sporadic abortions.[2,6,12] Clinical EVA has an incubation period of 3 to 14 days followed by fever (39–41°C), leukopenia, a serous nasal discharge, conjunctivitis, lacrimation and purulent ocular discharge, keratitis, palpebral edema, photophobia, and skin rash, commonly on the neck. Petechiae of nasal mucosa and conjunctivae may occur, along with moderate to severe anorexia and depression, weight loss, and in severe cases, abdominal pain and diarrhea. Respiratory signs, coughing, and dyspnea are seen less frequently. Edema of limbs, especially the hindlegs, and ventral abdomen, scrotum, and prepuce occurs. Secondary bacterial invasion is manifested by rhinitis and infection of the respiratory tract. Naturally infected horses usually have uneventful recoveries, and death is usually confined to experimental infection following dehydration, muscle weakness, and prostration. The course of infection is usually 3 to 9 days, and recovered horses have prolonged immunity.[1–3,5,6,12]

Abortion usually occurs within 7 to 10 days after the onset of maternal illness. The aborted fetus is often autolytic but may be fresh. Most abortions occur between 5 and 10 months' gestation and the prevalence of abortion is up to 50%. It is estimated that 40% to 80% of EVA-infected pregnant mares abort or deliver stillborn foals.

LESIONS

Gross lesions in horses with clinical EVA are mainly due to hemorrhage and edema from generalized vascular necrosis and include petechiae of the upper respiratory tract, lungs, gastric mucosa, adrenals, and all serous surfaces. Hemorrhagic enteritis is present and is usually more severe in the large intestine. Hemorrhage and infarction of the spleen are also observed. There is moderate to severe pulmonary edema, severe hydrothorax and hydroperitoneum, and edema of the mesentery and connective tissue and often of the intestinal wall, eyelids, limbs, ventral abdominal wall, prepuce, and scrotum.[1–3]

Characteristic microscopic lesions occur in the media of small muscular arteries in many organs, especially the intestine and adrenals. There is multifocal necrosis of myocytes in the media with fibrinoid replacement, edema of the vessel wall and adventitia, and infiltration of leukocytes, mainly lymphocytes. Thrombosis may occur in the intestine and lungs with infarction commonly occurring in the intestine, particularly the cecum, the colon, and the spleen. Massive necrosis occurs in lymph nodes and adrenals.[1–3,14]

Acute multifocal necrotizing myometritis occurred in the uterus of experimentally infected mares and may occur in the absence of generalized vasculitis.[14] The lesion is more severe in the internal part of the inner circular layer of the myometrium. The placentae were hyperemic but the blood vessels were histologically normal.[14]

Aborted fetuses may be fresh or autolyzed, and gross and microscopic lesions are rarely observed, although EVA virus may be recovered.[1–3,12,14] Lesions have, however, been observed in a few aborted fetuses and their membranes and included necrotizing vasculitis, especially of allantochorion, and inflammatory foci in various fetal organs.[4]

DIAGNOSIS

Clinical EVA is more severe than the other respiratory diseases and needs to be differentiated from equine herpesvirus infections, equine influenza, equine infectious anemia, and possibly leptospirosis by complement fixation, serum neutralization, and enzyme-linked immunosorbent assay tests.[7,10,12] Characteristic histopathologic changes can be confirmed by immunofluorescence.

The EVA virus can be isolated in equine kidney and rabbit kidney cell cultures.[1,12] Specimens for isolation include nasopharyngeal swabs, heparinized blood, urine, lung and spleen of aborted fetuses, allantochorion, and semen of affected stallions.[6,12,15] EVA is a significant cause of equine abortion and can present a diagnostic dilemma if respiratory signs are minimal.[5,12] Equine herpesviral, bacterial, and fungal causes should be eliminated by serologic and cultural procedures. Definitive diagnosis of EVA abortion can be provided by immunofluorescent tests on aborted fetal tissues and by serum neutralization tests on sera of infected aborting mares.

CONTROL

Sufficient data and an effective live, attenuated vaccine[8,16] are available for prevention and control of EVA.[6] Stallions should be vaccinated annually before the breeding season. Infected virus-shedding stallions can be mated to seropositive or vaccinated mares, provided that strict isolation is maintained between infected and noninfected horses. Mares should not be vaccinated during the last 2 months of pregnancy.[6] Vaccination of foals from nonimmune mares results in good protection, and foals from seropositive mares should be vaccinated at 6 months of age.

Kentucky instituted an effective EVA control program in 1985 based on serologic testing of stallions and mandatory annual vaccination of Thoroughbred stallions.[6] Carrier stallions are kept isolated and bred only to mares that were vaccinated more than 3 weeks previously or are EVA seropositive; the bred mares are isolated from

unvaccinated or seronegative horses for 3 weeks. The same general principles and hygiene for handling equine herpesviral abortion should be used for EVA abortions.

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CHAPTER 24

Bacterial Diseases of the Fetus and Placenta Associated with Fetal Loss in the Mare

T. W. SWERCZEK

Fetal lesions and placentitis associated with abortions and stillbirths are major causes of economic loss in mares in many countries throughout the world.[1–4] Before the mid-1980s the majority of pathogenic bacteria associated with equine placentitis and abortions were reportedly due to a group of bacterial microorganisms including *Streptococcus zooepidemicus, S. equisimilis* and other streptococci including non-beta-hemolytic *Streptococcus* spp., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Actinobacillus equuli.*[1,2,4–6] However, since the late 1980s and early 1990s other microorganisms have dramatically emerged as very significant causes of fetal loss in mares.[7–9] These agents include leptospires, nocardioform actinomycete group of organisms,[10] and microorganisms associated with early and late fetal loss in mares.[11] This chapter will review the emerging bacterial diseases currently causing equine fetal loss in mares.

The most common bacterial group currently being diagnosed is categorized as a group of organisms belonging to the nocardioform actinomycetes, of which there are several,[12] followed by the streptococcal group of bacteria including *Streptococcus zooepidemicus, Streptococcus* spp., and *Streptococcus equisimilis*. Other commonly isolated bacteria include *E. coli, Pantoea* spp., *Pseudomonas* spp., and *Leptospira* spp. In addition, several less commonly isolated bacteria are associated with fetal septicemia. The largest number of fetal losses are free of known pathogens, but are infected with a group of bacteria referred to as nonpathogens and saphrophytes.[11]

unvaccinated or seronegative horses for 3 weeks. The same general principles and hygiene for handling equine herpesviral abortion should be used for EVA abortions.

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Most gross and microscopic lesions affecting allantochorion fall into three categories: diffuse placentitis associated with a hematogenous origin, an ascending placentitis originating from vaginal tract and cervix, and a focal mucoid placentitis located in the anterior body of unknown origin.[13] It is not uncommon to find overlapping of placental lesions when multiple etiologic syndromes simultaneously affect the fetus. In the umbilical cord and amnion, the primary lesions are edema and hemorrhage, and secondary inflammation apparently is associated with secondary opportunistic and pathogenic bacteria. Seemingly, lesions in umbilical cord and amnion are emerging as a significant cause of fetal death associated with midterm and late-term fetal losses.

LEPTOSPIROSIS

Leptospirosis is a zoonotic bacterial disease of worldwide distribution that infects many species, including humans. In horses as well as other species, clinical responses range from inapparent to severe infections. Pregnant mares exposed to infected animals may abort.[14–16] Most infections are caused by leptospires in the Pomona serogroup, and less frequently by the servor grippotyphosa, and rarely other strains.[1,7,18–20] The horse is seemingly an incidental host for these and other serovars, with the possible exception of bratislava.[15,16]

Fetal Lesions

The most dramatic pathologic changes in the fetus are seen in the liver, which is enlarged, mottled, and pale to yellow. The microscopic changes consist of hepatocellular dissociation, hepatocellular vacuolar degeneration, and focal to multifocal fibrosis and necrosis of individual hepatocytes and Kupffer cells. There is intrahepatic bile stasis. Some of the hepatocytes develop into giant hepatocytes with abundant cytoplasm and binucleated to multinucleated nuclei. There is a mixed leukocytic infiltration in the portal trials.[21]

The kidneys are grossly enlarged and edematous with pale white radiating streaks in the cortex and medulla. The microscopic lesions consist of multiple focally extensive microabscesses with few to several giant cells, and multifocal nonsuppurative interstitial nephritis. The tubules may be dilated.[21]

The changes in the lungs and heart are occasionally present and include varying degrees of bronchopneumonia and subpleural, interlobular, and alveolar hemorrhages. The pneumonic changes are usually minimal to mild and consist of focal neutrophilic infiltrations. The microscopic changes in the heart are occasionally found and consist of multifocal infiltrations of mixed leukocytes in the myocardium. The lymphoid organs may have a mild lymphoid necrosis with neutrophilic infiltration and the reticuloendothelial cells proliferate in the sinuses.[21]

Placental Lesions

The placental changes occur in the allantochorion where there are patchy areas of necrotic mucoid exudates on the chorionic epithelium. There may be lesions of nodular cystic hyperplasia in the allantois. Microscopic changes in the chorion consist of thrombosis, vasculitis, mixed inflammatory cell infiltration of the chorion, and villous necrosis and calcification. There is cystic adenomatous hyperplasia of allantoic epithelium.[9,21] The spirochetes can be demonstrated in the majority of cases with immunofluorescent microscopic examination and in histologic sections stained by the Warthin-Starry method for spirochetes.[9,21,22]

For serologic testing, heart blood and pericardial fluid are ideal for testing fetuses and stillborn foals, and blood from the aborting mares can be tested for leptospiral antibodies by the microscopic agglutination test (MAT). The most common serovar found in affected fetuses and aborting mares are *Leptospira interrogans* serovars *pomona, grippotyphosa, copenhageni, hardjo, canicola, and bratislava*. The majority of cases react to only one serovar, but a few cases show cross-reactivity to more than one serovar.[9,21,22]

Epidemiology

Horses appear to serve as maintenance host for the serovar *bratislava* due to the high seroprevalence of *bratislava* antibodies in various horse populations throughout the world.[15] If in fact serovar *bratislava* is host adapted, transmission would be by direct contact with infected urine or by the venereal route from infected horses. *Bratislava* may be localized in the kidneys of horses and may be shed in the urine for the duration of the horse's life.[15]

In North America most horses seemingly are infected with serovars maintained in other animal species, and mares are considered incidental hosts. In the central Kentucky area and likely other areas in North America, *kennewicki, grippotyphosa,* and *hardjo,* found in skunks, raccoons, and cattle, respectively, are the nonhost-adaped serovars associated with equine abortions. [10] These species are commonly found on or around horse farms. Once horses are infected on a farm, horse-to-horse transmission is likely to occur as infected mares may shed high numbers of the nonhost-adaped leptospires for several weeks.[10]

Prevention and Control

Attempts should be made to prevent pregnant mares from coming into direct contact with the urine of maintenance hosts as well as indirect contact with contaminated environmental drinking water, feed, and bedding contaminated with urine from maintenance hosts. Various antibiotics have been used to treat infected and carrier mares with mixed results with apparent success in some mares, but not in others.[10]

MUCOID PLACENTITIS (NOCARDIFORM PLACENTITIS)

Bacteria that induce lesions of mucoid placentitis are categorized as a group of organisms belonging to the nocardioform actinomycete strains of bacteria, of which there are several [12] The causative organisms associated with these lesions are gram-positive, non-acid-fast, filamen-

tous bacteria. The bacteria were previously deemed to be nonpathogenic when they were found in lesions of mucoid placentitis. However, when the numbers of cases became more numerous in the early 1980s[23,24] and subsequently increased dramatically in the late 1990s, these previously called nonpathogenic bacteria are now considered emerging opportunistic pathogens and a significant cause of abortions and premature foals.[10]

Mucoid placentitis is a chronic form of placentitis that is focally extensive, affecting the junction of the horns and body of the allantochorion. Typically, the exudate on the surface of the chorion is mucoid and mimics a mycotic placentitis. Unlike mycotic infections of the placenta, the cervical area of the allantochorion is not affected, which suggests a hematogenous route of infection. The causal organisms induce extensive lesions on the chorionic surface that result in a mild to marked placental insufficiency, depending on the extent of the placental lesions. Affected fetuses either abort, or are born prematurely and typically resemble a twin due to the placental insufficiency. Affected fetuses are usually alive when aborted and lesions are restricted to the liver, which is enlarged and discolored due to the placental insufficiency.

Unlike mycotic organisms,[25] the causative bacteria are not easily demonstrated upon histopathologic examination with hematoxylin and eosin (H&E) staining, but can be demonstrated with the Gram stain.[24] The organisms are easily cultured on blood agar using routine bacteriologic techniques but may require more time to grow than other bacteria or contaminating organisms.[10,26]

Using molecular techniques and comparing the sequence of the 16S rDNA gene against the public databases indicated a relationship to members of the suborder Pseudonocardineae. One of the causative organisms has been further classified as *Crossiella equi*.[27] Other nocardioform bacteria, recently classified as *Amycolatopis* spp., are less commonly found in affected placentas.[28,29] It is likely that other similar nocardioform bacteria are also associated with mucoid placentitis lesions.

Also inducing lesions of mucoid placentitis is a bacterium identified as *Cellulosimicrobium (Cellumonas) cellulans* (formerly *Oerskovia xanthineolytica*).[30,31] This bacterium was first reported as causing abortion in Australia[31] and later in Kentucky.[30] Fetuses affected with *C. cellulans* have lesions in the chorion similar to those caused by *Crossiella equi,* but unlike *C. equi,* in some cases the lesions affect the cervical star area of the chorion, although in other cases the cervical star is not affected.[30] The chorionic epithelium is necrotic, thickened, and tan, and sharply demarcates the normal adjacent chorionic epithelium. The lesions in the body of the placenta are focally extensive, irregular, and very demarcated from adjacent normal chorionic epithelium. The villi in the center of the lesion are absent and covered by a brown, mucoid exudate. The amnion may be discolored yellow.[30,31]

The microscopic changes in the chorionic epithelium include short, blunted villi lined by hypertrophic trophoblastic epithelial cells. There is an infiltration of large numbers of lymphocytes and plasma cells, and fewer numbers of macrophages and neutrophils. There is an increase in vascularity and fibroplasia in the affected area of the chorion. There may be a squamous metaplasia of the trophoblastic epithelial cells. The chorionic surface is frequently covered with an amorphous eosinophic material that contains neutrophils and bacteria. Also, lesions may be present in the allantois and amnionic portion of the umbilical cord, but are less severe than the chorionic lesions and consistent with a nonsuppurative inflammation.[30]

The placental lesions in the chorion resemble those found with *Crossiella equi,* except that the cervical star area may be affected. In addition, fetuses with *C. equi* infections generally have no lesions, other than enlarged pale livers.[10] Fetuses infected with *C. cellulans* are usually emaciated and have gross lesions in the lungs and occasionally the liver. The lungs are firm and mottled red-tan. The liver is enlarged and mottled and may contain multiple 1- to 5-mm pale foci visible on the capsular surface and in some cases distributed throughout the hepatic parenchyma. The microscopic lesions in the liver are multifocal and there are mixed inflammatory cells in the portal triads.[30,31]

The microscopic lesions in the lungs include cellular debris, granulocytes, macrophages, and multinucleated histiocytic giant cells in the lumens of the alveoli and bronchioles. The pneumocytes are frequently hypertrophied, and the alveolar walls may be infiltrated with mononuclear inflammatory cells. Low numbers of gram-positive bacillary to filamentous bacteria can be seen in the pulmonary cells and in the cytoplasm of the multinucleated giant cells.[30,31]

The syndrome is not new, as it was first reported in 1982 in Australia.[31] Earlier, fetuses with identical lesions were seen in an outbreak of abortions on a Thoroughbred farm in central Kentucky in December 1970 (Swerczek, TW, unpublished data). Multiple abortions occurred in a herd of 25 pregnant mares. Two 10-month-old fetuses were submitted for necropsy examination, and the gross and microscopic lesions in the chorion, amnion, umbilical cord, lung, and liver were consistent with those subsequently described in 1982 in Australia[31] and in 2004 in Kentucky.[30] Multiple mixed bacteria were isolated from the placentas and fetuses. Gram stains of lungs with fetal pneumonia revealed bacteria in the alveolar macrophages and multinucleated giant cells that were morphologically consistent with those reported in fetuses in 1982[31] and 2004.[30]

There were no other fetuses with similar lesions submitted for necropsy examination during the 1970 and 1971 foaling season from other area farms (Swerczek, TW, unpublished data). An unconventional feeding program was given to the affected pregnant mares that aborted. The affected mares were being fed haylage starting in November 1970, and approximately 3 weeks later mares aborted. It was suspected that the causative bacterium may have been present in the haylage that was being fed to cattle as well as the pregnant mares on the farm. The farm did not experience similar abortions in subsequent years, but because haylage was the suspected as harboring the bacterium at that time, it was no longer fed after the mares aborted in the fall of 1970.

The source of infection of *Cellulosimicrobium (Cellumonas) cellulans* is most likely the environment. In

light of the cases in which haylage was suspected as early as 1970, these bacteria that are commonly found in soils may be also associated with the oral consumption of the causative bacteria in forages.

Prevention and Control

Until more is known on the distribution of the group of causative organisms in the environment, predisposing factors, and how these bacteria enter the reproductive tract of pregnant mares no recommendations can be made.

EARLY AND LATE FETAL LOSS SYNDROME

History

A dramatic spike in fetal losses, commonly referred to as mare reproductive loss syndrome (MRLS), was first recognized as a significant cause of fetal loss in the spring of 1980 and 1981 in the central Kentucky area.[32] However, retrospective analysis of breeding and pathology records of cases from area farms revealed similar losses to a much lesser extent in the late 1970s (Swerczek, TW, unpublished data). Many mares that were bred in February and March in 1980 later aborted in May and June. During May and June of 1980 late-term mares also aborted at the same time mares early in pregnancy aborted.[32] At that time, unusual climatic and environmental conditions occurred, including severe late spring frosts, which damaged lush grass and clover pastures at late as May 9, 1980. The syndrome was also seen during the 1981 foaling season, but to a lesser extent. During the subsequent years the syndrome was seen each spring, as well as the fall of the year (Swerczek, TW, unpublished data), but there were no drastic spikes in abortions until the breeding season of 2001 when massive early and late fetal losses occurred similar to 1980, but to a much greater extent.[33–36] Again in 2001, climatic and environmental conditions, similar to those that occurred in 1980 and 1981, occurred but to greater extremes. A series of late frosts and freezes affected area pastures during the late spring. Also, extremes of unseasonal high climatic temperatures occurred before and after the late severe frosts and freezes for 1 week starting on April 17, 2001.[37]

The abortions seem to cluster during a brief period of time in the late spring seemingly associated with simultaneous environmental exposure.[33–35,37] Risk factors associated with late-term abortions include increased amount of time at pasture and less time in the stall; feeding concentrates and hay, especially alfalfa, on the ground; access to pasture after midnight during the 4-week period prior to abortions; and drinking water from a water trough or not having access to water buckets or automatic water devices. It was concluded that exposure to pasture during critical environmental conditions similar to those seen in 1980 and 1981 seem to predispose mares to having late-term abortions and stillborn foals.[33,34]

Abortions seemingly occur 9 to 10 days after frost or freeze to lush grass and clover pastures in the late spring and early fall.[37] Retrospective analysis of pathology records between 1980 and 2001 also revealed that spikes of abortions also occurred 9 to 10 days following severe frost and freezes to pasture forages in the late spring and fall (Swerczek, TW, unpublished data). It was found that mares that had access to pasture after midnight were more likely to abort.[34] This was likely associated with frosts and freezes to pastures during the early morning hours when temperatures are the lowest of the day and when frosts and freezes to pastures are most likely to occur in the late spring. Potassium will increase in forages during the early morning hours and will spike during and after a frost.[38] It is suspected that high-potassium frost-damaged forage may be involved in the pathogenesis of the abortions. The high potassium in affected forages would favor the overgrowth of endophytic and saprophytic bacteria in frost-damaged forages. Also, sugars, primarily fructans, will increase in frost-damaged cool season grasses and forages.[37,38] Increase in sugar levels, in addition to elevated potassium levels, would dramatically favor the overgrowth of endophytic and saprophytic bacteria, many of which are found in fetuses and placentas of affected aborted fetuses.

Bacterial Findings

The most common bacteria isolated from aborted fetuses include non-beta-hemolytic *Streptococcus* spp., followed by an *Actinobacillus* species and numerous other bacteria classified as nonpathogens.[11] In addition, plant bacterial endophytes, including *Microbacterium* spp., *Cellumonas* spp., and *Proteoa* spp. were commonly found in affected fetuses (Swerczek TW and Allen GP, unpublished data, 2001–2005). It is suspected that these endophytic plant bacteria, which are present and increase in numbers in stressed pasture forages, may be associated with the pathogenesis of the early and late fetal loss syndrome.[37]

Fetal and Placental Lesions

In early fetal losses there are no characteristic inflammatory lesions. Bacterial are present in the placental membranes and fetal tissues. In midterm fetuses, inflammatory lesions may be present in the amnion and amnionic portion of the umbilical cord. In midterm affected fetuses, the fetus may die from a twisted cord caused by the ongoing developing lesions in the amnionic portion of the umbilical cord. In late-term fetuses there may be a unilateral or bilateral hemorrhagic endophthalmitis. The lungs contain squamous epithelial cells in the alveoli with or without concurrent infiltration of inflammatory cells including neutrophils, macrophages, and monocytes in the alveoli and interstitium. In the heart there may be a focal myocarditis and pericarditis. The placental lesions are characteristic in late-term fetuses and in placentas from foals that are affected but do not abort and are born alive. There is a hemorrhagic, edematous amnionitis and funisitis affecting primarily the amnionic umbilical cord, and to a lesser extent the allantoic umbilical cord and the surface of the allantoic portion of the allantochorion (Swerczek, TW, unpublished data). The chorionic epithelium is usually free of lesions. However, occasionally, there are mixed bacterial infections with

concurrent bacteria, like *Cellulosimicrobium cellulans,*[30] and in these cases the chorionic epithelium is also affected.

In late-term fetuses, like midterm affected fetuses, the amnionic portion of the umbilical cord often becomes constricted and sacculated. The affected umbilical cord may become excessively twisted owing to the ongoing inflammatory lesions, and the fetus may die because of circulatory compromise to the developing fetus (Swerczek, TW, unpublished data).

The early and late fetal loss syndrome is not restricted to mares. At the same time mares were aborting during the spikes in abortions in 2001, other herbivores were also aborting, including cattle, goats, sheep, llamas, and buffalos. Abortions in cattle were seen after pregnant cows consumed haylage harvested after the forage was severely affected by frost and freezes. Affected bovine fetuses had the same lesions of hemorrhagic and edematous amnionitis and funisitis affecting the amnionic umbilical cord, as were present in affected equine fetuses. Myocarditis and endophthalmitis were also present in bovine fetuses. In bovine fetuses, like equine fetuses, plant bacterial endophytic bacteria, including *Microbacterium* spp., *Cellumonas* spp., and *Proteoa* spp. were commonly found in affected fetuses (Swerczek TW and Allen GP, unpublished data, 2001–2005).

Prevention and Control

The early and late fetal loss syndrome seemingly is associated with environmental factors and conditions that affect pasture forages.[34] The use of the terms early- and late-term fetal loss syndrome seemingly is a misnomer. Rather than the stage of pregnancy, abortions are associated with simultaneous environmental exposure to an unknown factor(s) during certain times of the year, usually spring and fall. However, the majority of pregnant mares are either in early or late gestation in the late spring when spikes of abortions are more likely to occur. Fetal losses also occur at midterm, usually in the fall of the year.

Epidemiologic studies suggest that the amount of time, especially after midnight, pregnant mares spend in pastures during periods of environmental and climatic stress are risk factors for fetal losses.[34] Until more is known about the specific etiologic factor(s) in pastures and forages, no preventive measures can be suggested.

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CHAPTER 25
Fungal Abortion

T. W. SWERCZEK and STANLEY M. DENNIS

Fungi are important causes of sporadic abortion and are responsible for up to 10% of reported abortions in mares.[1,2] The fungi include *Aspergillus fumigatus, Mucor* spp., and *Allescheria boydii,* with *Aspergillus* being the most common.[2–5] Rarely reported fungi include *Coccidioides immitis,*[6] *Cryptococcus neoformans, Candida albicans,* and *Histoplasma capsulatum.*[2]

MATERNAL SIGNS

Mares may display signs of estrus early in pregnancy and abort later at 8 to 11 months of gestation, usually without any premonitory signs.[7] Infected mares may run milk for days before abortion and occasionally have a thick, brownish, adherent vulval discharge.[1] A purulent vulval discharge may be evident for some days after abortion but resolves spontaneously.[1] Infection with *Aspergillus* and *Mucor* spp. does not persist in the uterus and does not interfere with subsequent fertility.

PATHOGENESIS

Although fungal invasion of the pregnant uterus may be hematogenous or transcervical, it primarily ascends from the vagina via a patent cervix. Initially, infection begins in the chorion adjacent to the cervical star, then progresses cranially up the uterine body.[1,2,7] The resultant chronic ascending chorionitis causes progressive placental insufficiency that interferes with fetal nutrition and growth and results in intrauterine growth retardation.[1] Infection eventually results in abortion or premature expulsion of a dead or nonviable fetus.

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FETAL MEMBRANE LESIONS

Grossly, the affected chorioallantois is diffusely involved; is yellowish, thickened, and leathery; and is clearly demarcated from the normal, reddish chorioallantois.[7,8] The chorionic surface is necrotic and usually covered with thick, adherent, mucoid exudate.[9] The amnion may have irregular necrotic plaques in about 10% of *Mucor* spp. infections.[1,10] Amnionitis is not normally associated with aspergillosis.[3] Microscopically, there is marked squamous metaplasia and widespread necrosis of chorionic villi and numerous hyphae are present.[3,4,7]

FETAL LESIONS

Fetuses aborted because of mycotic placentitis are usually fresh, emaciated, and small for their gestational age.[3,7] Affected fetuses are usually aborted dead but may be expelled alive but nonviable. Scattered areas of mycotic dermatitis are rare.[11] The liver is usually pale, enlarged, and mottled.[7] Grayish white granulomas, 1 to 3 mm in diameter, scattered throughout the lungs are not common and are associated with *Mucor*-induced amnionitis.[10] Microscopically, the liver has fatty change, portal hepatitis, and bile ductule proliferation.[7] Multifocal, granulomatous pneumonia with giant cells, macrophages, and hyphae is present in fetuses with grayish white pulmonary granulomas.

DIAGNOSIS

The specimen of choice for diagnosing mycotic abortion is the chorioallantois; it should be submitted to the diagnostic laboratory fresh and in 10% buffered neutral formalin.

Diagnosis of mycotic abortion is confirmed by demonstrating fungal hyphae by smears, histopathologic examination, or culture. Lesions of chronic mycotic placentitis must be differentiated from chronic bacterial placentitis, especially streptococcal.

CONTROL

Control depends on good management and hygiene procedures. Care must be taken to avoid the use of moldy hay and straw. Aborting mares should not be bred on foal estrus.

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CHAPTER 26

Evaluation and Management of High-Risk Pregnancy in the Mare

CHARLES T. ESTILL

DEFINITION

A high-risk pregnancy is one in which a condition or multiple conditions exists that are potentially serious or life threatening to either the mare or foal.[1] Those conditions may be the culmination of physical, infectious, endocrinologic, or behavioral abnormalities. Maternal conditions are generally more easily recognized and assessed than fetal abnormalities[1] and may either be limited to the genital tract or involve any other body system such as the gastrointestinal tract or musculoskeletal system. Not only is fetal well-being generally difficult to evaluate but potentially helpful intervention is limited.

PRESENTING CLINICAL SIGNS

The majority of high-risk pregnancy cases are presented because the owner observed a vaginal discharge, noted premature mammary gland development and lactation, or suspects twin pregnancy, or because there is a serious medical or surgical condition in a pregnant mare. Mares with larger than normal abdominal size or overdue pregnancies should also be considered high-risk pregnancies. Mares in which fescue toxicosis is known or suspected are frequently presented for lack of mammary development in late gestation or for prolonged gestation. In other situations there may not be any clinical signs to indicate a mare is experiencing high-risk pregnancy other than historical information from previous pregnancies or parturitions.

OBJECTIVES OF INTERVENTION

In nearly all cases, the primary objective of managing a high-risk pregnancy should be to allow the foal to develop in the uterine environment as long as possible. This objective may be accomplished by: providing antimicrobial therapy when indicated, promoting uterine quiescence, countering the adverse effects of endotoxin, and maintaining adequate fetal gas exchange and nutrition. In mares with an underlying medical or surgical condition, the ideal objective of intervention should be to correct the condition in order that the mare will be able to carry the pregnancy to term. Therefore, the clinician's goal is to maintain the intrauterine environment in a state as close to normal physiologic health as possible. Extrauterine survival of premature foals is unlikely because the final stages of fetal maturation necessary for extrauterine life occur within the last 5 days of in utero development.[2] Even an abnormal uterine environment is often more conducive to maintaining the life of the foal than neonatal intensive care. Premature delivery is indicated only in situations in which extrauterine survival is more likely than continued survival in utero. Exogenous corticosteroids are not effective for induction of fetal maturity owing to their inability to cross the placenta. However, foals that are chronically stressed in utero have the ability to respond by maturing precociously and may be delivered after a relatively short gestation but thrive and develop normally after birth.

ASSESSMENT OF FETAL WELL-BEING

Clinicians presented with sick, pregnant mares must determine if the fetus is affected, how severely it is affected, and the stage of fetal physiologic maturity.[3] Clinical procedures for determination of fetal condition and maturity include palpation per rectum, transrectal and transabdominal ultrasonography of the fetus and placenta, examination of the vagina via speculum, serial blood sampling for hormone assay, amnio-/allantocentesis, fetal electrocardiography, and measurement of mammary secretion electrolytes. Guidelines for an equine biophysical profile based on fetal ultrasonography are available. A low score is a definite indication of an impending negative outcome; however, a high score does not assure a positive outcome.[4]

Palpation per rectum may be used initially to determine fetal viability. A foal with spontaneous movement can be considered alive, but one must bear in mind that stressed foals may demonstrate frequent bouts of hyperactivity and normal foals have periods of intermittent quiescence. Therefore, an unresponsive foal should not be immediately declared nonviable but must be assessed further by repeated examination per rectum or by ancillary procedures described in the paragraphs that follow.

Transabdominal ultrasonography is useful for evaluating fetal position, size, heart rate and rhythm, aortic size, fetal breathing movements, fetal fluid depths and echogenicity, uteroplacental thickness, and fetal move-

ment. Because of the large variation in normal fetal heart rates, fetal heart rate monitoring is not a particularly sensitive method to determine well-being. Fetal heartbeat is easily evaluated by transabdominal ultrasonography and can be monitored continuously via a Doppler heart rate monitor,[5] but fetal electrocardiography is widely available and is more suitable for long-term monitoring. An electrocardiographic (ECG) lead is placed just in front of the mare's udder and another lead is placed on her back in the lumbar area. The fetal tracing is recognized superimposed on the maternal tracing by its rapid rate and low amplitude. In normal equine pregnancies, fetal heart rate decreases progressively with advancing gestation and has a regular rhythm. Prior to day 160 of gestation fetal heart rate exceeds 120 beats per minute but gradually decreases to 60 to 80 beats per minute just prior to parturition. Periodic accelerations are generally associated with fetal activity and suggest fetal health.[6] The frequency of cardiac accelerations associated with fetal activity increases with gestational age.[6] Normal fetal movement can usually be detected a minimum of every 5 minutes. It is normal for heart rate to increase to up to 110 beats per minute for short periods of time in association with fetal activity during late gestation, but persistent tachycardia is pathologic and associated with maternal fever or dehydration, fetal hypoxia, or fetal/placental infection.[6] Bradycardia is an indication of fetal asphyxia. Fetal bradycardia may be present as an adaptive heart rate pattern suggesting either cardiovascular efficiency or early stage hypoxia.[6] The fetus adapting to hypoxic stress will have a slow heart rate and appear inactive with no fetal breathing and few spontaneous movements and cardiac accelerations. If the foal fails to adapt, tachycardia develops in attempt to maintain perfusion and there is no concomitant increase in fetal movement. Finally, with progressive disease, as the myocardium becomes affected, terminal bradycardia may be observed.[6] The disappearance of beat-to-beat variation is an ominous sign. The clinician should be aware that maternal medications, such as detomidine or butorphanol, transiently reduce fetal heart rate variability.

Placentitis, the cause of nearly one third of all equine abortions, is associated with an increase in uteroplacental thickness (Fig. 26-1). Mares with placentitis rarely show signs of systemic illness. Ultrasonographic evaluation per rectum is the established method for measuring uteroplacental thickness. Measurement is made just cranial to the cervix on the ventral wall of the uterus. In normal pregnancies, the combined thickness of the uterus and placenta (CUT) does not change between 4 and 8 months of gestation, but increases significantly each month between 10 and 12 months of gestation.[7] A CUT greater than 1.5 cm at any time (normal is 1.0–1.2 cm at term) is direct evidence of placental or uterine edema and is usually due to placentitis. Separation of the allantochorion from the uterus with accumulation of hyperechoic (purulent) exudate is another indication of placentitis (Fig. 26-2). Large or progressively enlarging areas of placental detachment are abnormal and contribute to fetal compromise and eventual death.[8] Increased echogenicity of either amniotic or allantoic fluid can also be regarded as an indication of placental disease.

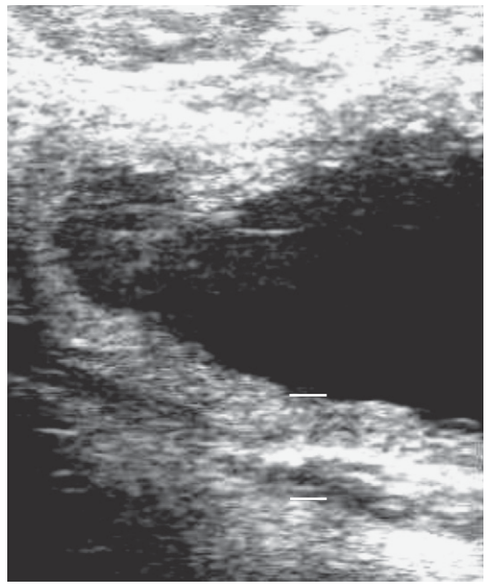

Fig. 26-1 Transrectal ultrasound image (5 MHz linear) demonstrating placental thickening associated with placentitis in a late-term mare. Uteroplacental thickness, as measured between the white bars, greater than 1.5 cm, is considered abnormal. (Courtesy MHT Troedsson.)

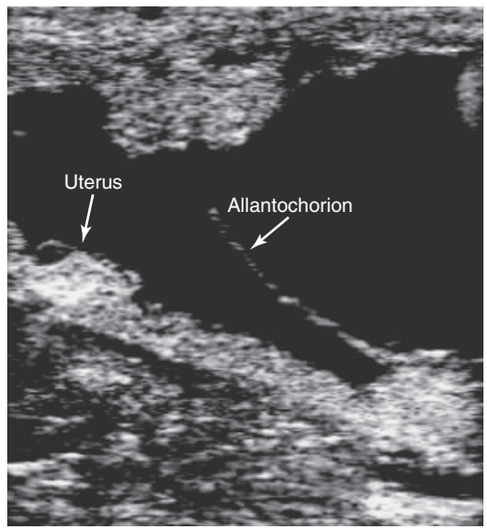

Fig. 26-2 Transrectal ultrasound image (5 MHz linear) demonstrating separation of the allantochorion from the endometrium in a mare. Mares with placental separation are treated similar to mares with placentitis. (Courtesy MHT Troedsson.)

Visual examination of the vagina and cervix is readily accomplished through a sterile cylindrical speculum. It should be performed in all mares with a vaginal discharge and in mares with premature mammary development and lactation. Cases of suspected placentitis should also be examined vaginoscopically. A careful examination provides information on the presence and character of uterine discharges as well as the competency and integrity

of the cervix. Culture specimens of discharges or placenta, which may be seen bulging through a partially dilated cervix, are readily obtained at the time of examination.

Serum hormone analysis can provide useful diagnostic and prognostic information. After about 90 days of gestation, maintenance of pregnancy is dependent on steroid hormones produced by the fetoplacental unit. The endocrine patterns in periparturient mares are characterized by increasing concentrations of progestagens and decreasing estrogens. These hormones are produced from precursors of fetal origin, metabolized by the fetoplacental unit or endometrium, and act primarily locally on the uterus. The mare is unusual in showing an increase rather than a decrease in circulating progestagens before delivery. Maternal serum or plasma concentrations of a number of progestagens, including 5α-dihydroprogesterone, escalate gradually in the last 3 to 6 weeks of gestation and peak 24 to 48 hours prior to parturition[9] (Fig. 26-3). This pattern is evidence of fetal maturity and readiness for birth because the source of the prepartum rise in progestagens is fetal adrenal steroidogenesis. Progesterone is often undetectable (<1 ng/ml) in plasma of late gestation pregnant mares but metabolites of progesterone (progestagens) are present in high concentrations.[10] Whether maternal serum progesterone concentrations are high or low in normal mares depends on the specificity of the testing procedure. Many test kits for progesterone cross-react with metabolites of progesterone such as 5α-dihydroprogesterone making interpretation of laboratory testing in high-risk mares difficult unless one is aware of the specificity of the testing procedure. The maternal endometrium metabolizes progesterone and pregnenolone to 5α-dihydroprogesterone.[11] A rise in maternal plasma progesterone concentration, when the foal is at least 290 days of gestational age, has been associated with birth of premature but viable foals. This scenario is most likely to occur in cases of slowly developing placentitis or partial premature placental separation, which stresses the foal but provides ample time for the foal to respond to the stress and mature precociously to the point at which extrauterine survival is possible. Induction of precocious maturity takes approximately 3 weeks. Abnormal trends in maternal plasma progesterone are associated with fetal compromise. Increased concentrations of plasma progesterone during mid- and late gestation are suggestive of placentitis; however, therapeutic decisions should not be made on the basis of a single sample, and serial determinations are required to detect a clinically useful trend.[12] Maternal plasma progesterone concentration determinations should be made at 48-hour intervals for a minimum of three sampling periods. Rising and elevated progesterone concentrations are associated with a good prognosis for fetal viability in late gestation. Consumption of endophyte-infected tall fescue grass may prevent the dramatic rise in progestagens prepartum.[13] A rapid and sustained decline of progesterone concentrations to less than 2 ng/ml for more than 3 to 4 days is associated with impending abortion. An investigation of hormonal profiles of pregnant mares treated either medically or surgically for colic revealed that progesterone concentrations in mares that subsequently aborted either dropped rapidly or were low at the time of admission and decreased to below 2 ng/ml.[14] If progestagens drop

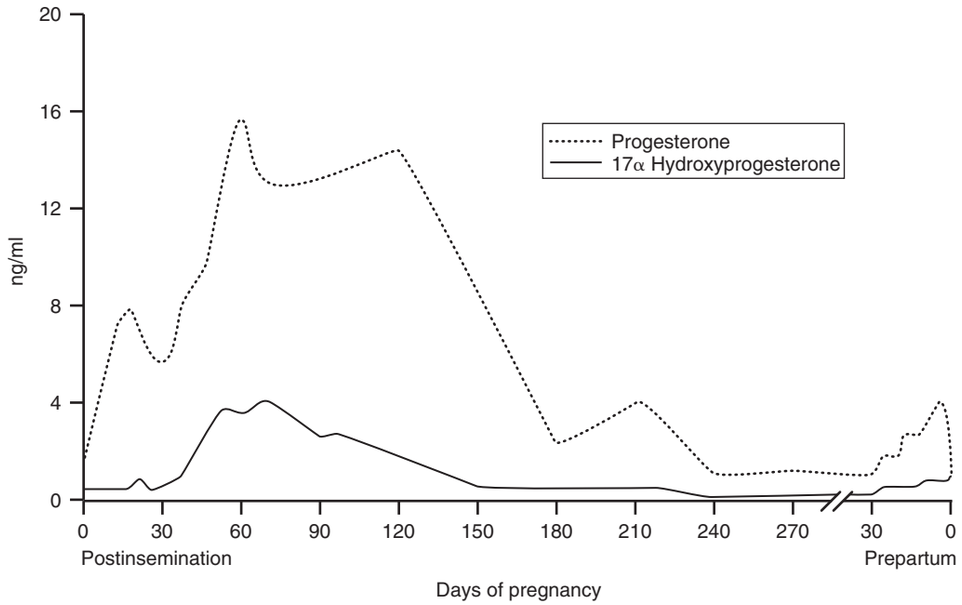

Fig. 26-3 Concentrations of progesterone and 17-hydroxyprogesterone during pregnancy in mares. Note the gradual rise in progestin concentrations during the last month of gestation, culminating in a sharp peak just before parturition. This rise is associated with maturation of the fetal adrenal glands and readiness for birth. (From Holtan DW, Nett TM, Estergreen VL: Plasma progestagens in pregnant mares, *J Reprod Fertil* 1975;Suppl 23:419.)

rapidly, owing to infection with equine herpes virus,[15] mares invariably abort.

The fetoplacental unit is the major source of estrone sulfate after day 70 of gestation. Maternal plasma concentrations are highest between 160 and 280 days of gestation. Concentrations decline during the last month concomitant with a reduction in size of the fetal gonads. Declining estrone sulfate concentrations prior to day 280 may be an indicator of fetal compromise but are of limited predictive value because of wide variation in concentrations in normal pregnant mares. Estrone sulfate concentrations are not a reliable indicator of pregnancy or fetal well-being in the last 2 weeks of gestation. Estrone sulfate concentrations after foaling or abortion may overlap the concentrations seen in the last month of gestation.[14] However, a series of estrone sulfate concentrations obtained from a sick pregnant mare may be useful in determining fetal well-being. A sharp decline in estrone sulfate concentration, which does not rebound within a few days, may be an indication of fetal compromise and help differentiate a viable fetus from one that has died in utero. On the other hand, estrone sulfate concentrations may not decline to nonpregnant levels until several days after fetal death so that it is not always possible to determine fetal viability on the basis of estrone sulfate concentrations.[15]

The assay of serum relaxin has been found to be a sensitive indicator of placental dysfunction in the late-term mare.[16] In a population of mares with normal pregnancies and deliveries, serum relaxin concentrations ranged from 45.0 to 85.0 ng/ml, with a mean value of 63.0 ng/ml during the last 7 weeks of gestation. Relaxin concentrations declined markedly in mares with placentitis during the same period (Fig. 26-4).[16] In the future, exogenous relaxin may be incorporated into the treatment protocol for placentitis in late gestation mares.

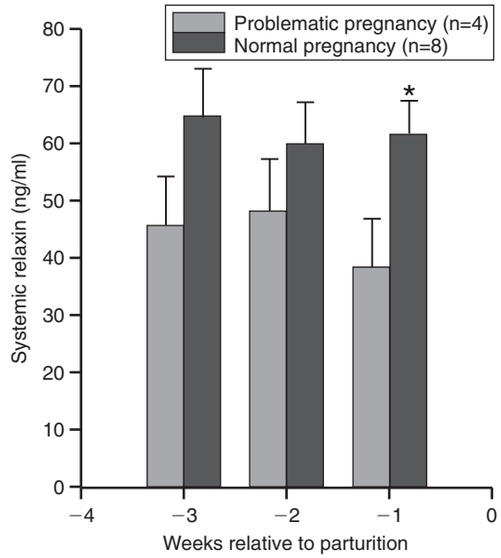

Fig. 26-4 Circulating relaxin in gravid pony mares grazed on endophyte-infected pasture and experiencing normal or problematic pregnancies associated with fescue toxicosis. Plasma relaxin was consistently lower in mares having pregnancy complications regardless of treatment. This difference was significant ($P = 0.03$) the week before delivery. (From Ryan PL, Bennett-Wimbush K, Vaala WE, et al: Systemic relaxin in pregnant pony mares grazed on endophyte-infected fescue: effects of fluphenazine treatment, *Theriogenology* 2001;56:471.)

Although amnio- and allantocentesis are not widely practiced in equine clinical medicine, in some instances valuable information may be obtained with these procedures. In cases of placentitis, fluid obtained by allantocentesis can be evaluated cytologically and cultured to determine infectious etiology and direct appropriate antimicrobial therapy. Experimental work indicated that analysis of phosphatidylglycerol concentrations in fluid obtained by amniocentesis may be useful for determination of fetal pulmonary maturity in the equine.[17] Unfortunately, pulmonary maturity standards have not been established for the equine, and there is a high degree of overlap between values obtained at various stages of gestation.[18] These procedures present some risks, particularly the introduction of bacterial contaminants into the allantoic or amniotic spaces. Another possible complication is tearing of the fetal membranes, permitting mixing of amniotic and allantoic fluid followed by abortion.

Measurement of prepartum mammary secretion electrolytes is a valuable diagnostic aid in determining fetal readiness for birth but must be interpreted with caution. Several commercial kits are available to the practitioner for measuring calcium concentrations in prefoaling mammary secretion. Dramatic changes in calcium, sodium, and potassium concentrations occur just days prior to parturition, and these changes appear to be correlated with fetal maturation.[19,20]

A progressive increase in mammary secretion calcium concentration normally occurs over the last 6 days of pregnancy, and parturition is associated with concentrations exceeding 40 mg/dl.[20] However, mares carrying normal pregnancies that were induced after mammary secretion calcium concentrations rose above 16 mg/dl delivered mature foals, and mares with concentrations less that 12 mg/ml produced dysmature foals that rarely survive.[20] Three to 5 days prior to foaling there is an inversion of the sodium and potassium concentrations such that potassium, which is initially significantly lower than sodium, becomes higher than sodium.[19] In a sick pregnant mare, mammary secretion calcium and potassium concentrations may rise prematurely due to disease-induced hormonal changes.[21] For this reason, in cases of high-risk pregnancy, the mammary secretion electrolyte concentrations cannot always be relied on to predict readiness for birth because fetal maturation may not be synchronous with maternal preparation for birth. Occasionally, when a mare recovers from the underlying disease the electrolyte concentrations return to values consistent with the stage of gestation. Udder development may regress and then resume as parturition approaches. For example, mammary secretion electrolytes and udder development may increase prematurely in cases of placentitis but then return to normal following successful treatment.

MANAGEMENT OF FETAL AND PLACENTAL DISORDERS

Placentitis is one of the few causes of high-risk pregnancy that may be amenable to treatment. Approximately 75% of placentitis cases are associated with *Streptococcus zooepidemicus*.[22] When possible, antimicrobials should be chosen based on sensitivity testing and administered systemically. However, trimethoprim sulfadiazine (25–30 mg/kg/day PO[23,24]) has been used empirically based on its ability to cross the placenta and enter fetal fluids and the fetus.[25] Potassium penicillin G (22,000 IU/kg every 6 hours IV) and gentamicin (6.6 mg/kg every 24 hours) may also reach therapeutic concentrations in fetal fluids.[26] Instillation of antibiotics directly into the allantoic space has not been widely tested but can be considered when culture and sensitivity results indicate that trimethoprim is unlikely to be effective. Antibiotics should be administered until the vaginal discharge and premature lactation cease.

Several other pharmacologic agents may be administered to mares with placentitis or other causes of high-risk pregnancy, but their effectiveness has not been conclusively demonstrated. Nonsteroidal anti-inflammatory agents have been administered to reduce prostaglandin (PG) production, although their effectiveness in altering the overall outcome has not been demonstrated. Experimentally, pretreatment with flunixin meglumine was effective in preventing endotoxin-induced PGF secretion and abortion[27] but is ineffective at modulation of uterine PGF production and prevention of endotoxin-induced abortion when given 1 or 2 hours after endotoxin administration and, therefore, cannot be considered reliable therapy for conditions that are likely to result in increased uterine PGF secretion.[28] Pentoxiphylline (8.5 mg/kg every 12 hours PO) can be added to the treatment regimen for its potential inhibition of proinflammatory cytokines.[29,30] Tocolytics may be useful in cases of endotoxemia or placentitis. Clenbuterol, due to its β_2-adrenergic agonist effect, is capable of inducing uterine relaxation. Experimental evidence suggests clenbuterol is ineffective at delaying parturition, at least in normally foaling mares, when given once a day.[31] Clenbuterol can be given to typical sized mares as an IV bolus (300 μg), which will provide uterine relaxation for 2 hours, or incorporated into an IV drip or for mares at high risk for impending abortion.[32] Low-dose aspirin therapy, as used in horses with laminitis, might provide protection for placental circulation by inhibiting platelet aggregation and vasoconstriction.[33] Synthetic progestins (altrenogest 0.088 mg/kg PO every 24 hours) may be used empirically to induce uterine quiescence and reduce PGF release[34] and for its possible effect of blocking cytokines.[35]

Chronic placental separation may occur concomitantly with placentitis and frequently involves a relatively small area in the vicinity of the cervical star. If the fetus is mature, induction of parturition should be performed. If the fetus is not mature, a mare with placental separation is treated as one with placentitis. In the future, protocols may be developed to induce precocious maturation of fetal foals by injecting the mare with adrenocorticotropin hormone (ACTH) or by intrafetal or intrauterine injection of betamethasone, ACTH, or corticotropin-releasing hormone (CRH).

MANAGEMENT OF MATERNAL DISORDERS

Although several maternal disorders may have an adverse influence on pregnancy, the equine fetus is amazingly resilient in the face of maternal disease. Some examples of maternal conditions that may potentially threaten fetal well-being include colic, endotoxemia, proximal enteritis, colitis, peritonitis, pleuritis, laminitis, chronic painful musculoskeletal disorders, fescue endophyte toxicosis, abdominal tunic hernia, uterine artery rupture, and dystocia. In general, a single episode of uncomplicated colic has no observable effect on the fetus. When colic is accompanied by endotoxemia or requires surgical intervention, abortion is more likely. Endotoxemia appears to have the greatest direct effects on the fetus during the last 60 days of gestation.[36] Mild to moderate medical colic did not appear to have an effect on fetal survival, but severe medical colic cases, especially when accompanied by endotoxemia, have a poorer prognosis for fetal survival.[36] In a retrospective analysis of 74 cases of surgical colic in pregnant mares, it was observed that the stage of gestation at initial examination, type of surgical lesion, duration of anesthesia, and degree of intraoperative hypoxia or hypotension was not associated with increased rate of abortion even though there was a 20% abortion rate overall.[36] However, severe intraoperative hypoxia (arterial oxygen pressure <80 mm Hg, repeatedly) during the last 60 days of gestation was associated with either abortion or delivery of severely compromised foals that failed to survive.[36] In early pregnancy (<60 days), endotoxemia may be associated with luteolysis and embryonic loss.[37] In mares experimentally infused with endotoxin, luteal activity was compromised within 9 hours.[37] Therapy for endotoxemia includes correction of the inciting cause, administration of cyclooxygenase inhibitors such as flunixin meglumine, and if the gestational age is less than 90 days, oral altrenogest.[35,38] Therapy can continue until the mare is greater than 90 days pregnant or endogenous production of progestins can be demonstrated.

When surgical intervention for colic is required in pregnant mares it should be performed as soon as possible after the decision is made. For mares undergoing surgery during the last 60 days of gestation, arterial oxygen should be maintained at 80 mm Hg or greater and adequate perfusion pressure maintained during anesthesia. Although no particular anesthetic protocol has been shown to be clearly superior, it has been recommended that intravenous anesthesia using a combination of detomidine, ketamine, and guaifenesin with oxygen administered via tracheal catheter be used and that anesthesia time be less that 2.5 hours. Alternatively, propofol, although very costly, may be safely used.

Pregnant mares presented for gastrointestinal disturbances are often fasted, a practice that may adversely affect pregnancy. Fasting mares for 24 hours caused a surge in PGFM concentrations as well as a dramatic decline in glucose and an increase in free fatty-acid con-

centrations in peripheral plasma.[39] Elevations in PGFM concentrations may be associated with adverse effects on uterine blood flow and myometrial activity, and prostaglandins may stimulate oxytocin release and the onset of uterine contractions. Refeeding the mares or administering glucose IV induces a rapid decline in PGFM concentrations.

Uterine artery rupture is usually associated with parturition but occasionally occurs prepartum. If hemorrhage is confined within the broad ligament control of pain is the mainstay of therapy. If hemorrhage escapes the confines of the broad ligament, the mare is likely to die acutely from hypovolemia. Therapy includes correction of hypovolemia by administration of fluids or whole blood, anticoagulant therapy, and pain management.

Hernias or rupture of the abdominal tunic are an occasional cause of high-risk pregnancy with rupture of the prepubic tendon the most common form. When a mare is observed to have a cranial displacement of the udder, it is an indication of previous prepubic tendon rupture. Pregnancy in these mares may either be aborted or an attempt made to manage the pregnancy to term, realizing that further damage may result and rebreeding would be very risky. The mare should be confined, given analgesics as needed, and have supportive abdominal wraps applied. Parturition should be induced when the criteria of fetal and maternal readiness are met so that delivery can be assisted.

SUMMARY AND CONCLUSIONS

High-risk pregnancy in the mare can result from a wide variety of maternal and fetal conditions. In nearly all cases, maintenance of the foal in the uterine environment is preferable to premature delivery of a compromised foal. Fetal maturation proceeds rapidly during the last 5 to 7 days of gestation, but the maturation process may actually begin about a month prior to parturition. Foals do have the ability to respond to intrauterine stress by maturing precociously in utero and may be delivered as viable foals after this process has taken place. Unlike other species, exogenous corticosteroids do not induce precocious fetal maturation in foals so reliance on the natural process generally results in improved likelihood of a favorable outcome.

Ultrasonography has greatly improved our ability to diagnose and monitor conditions that may compromise pregnancy in late-term mares. Development of fetal biophysical profile parameters has permitted clinicians to quantify fetal development and offer a prognosis based on repeatable measurements.[4,5] Evaluation of fetal fluids is a relatively new development in equine fetal perinatology and holds promise for more precise diagnostic and therapeutic intervention.[17,18] Assessment of readiness-for-birth using endocrine testing and prepartum mammary secretion electrolyte concentrations has allowed improved prediction of the likelihood of extrauterine survival.

Therapeutic intervention strategies are available to treat conditions such as placentitis that will permit maturation of a fetus to the point where extrauterine survival is possible. Pharmacologic compounds including hormones, tocolytics, antibiotics, antiinflammatory agents, and mediators of vascular permeability and cytokine activity all have a role in management of selected cases of high-risk pregnancy.

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CHAPTER 27
Induced Abortion

AMANDA C. RAGON

In mares, elective termination of pregnancy is performed for several reasons, including mismating, change in ownership, age or health of dam, abnormal gestation, and twin pregnancy. Many methods may be used to induce abortion, and care should be taken to select a procedure that is safe and effective and that minimizes damage to the mare's reproductive tract and future breeding health. When terminating pregnancy, the clinician should consider the following factors: stage of gestation, presence of endometrial cups, expected time of return to estrus, presence of twin fetuses, and physical condition of the mare. In every case of elective abortion, the patient should be re-examined at an appropriate time after the procedure to ensure that pregnancy has been effectively terminated.

INDUCED ABORTION BEFORE FUNCTIONAL ENDOMETRIAL CUPS (DAYS 0–33)

The earlier pregnancy termination is attempted, the more likely it is to be safe and successful; however, no method has been shown to reliably terminate pregnancy before

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INDUCED ABORTION BEFORE FUNCTIONAL ENDOMETRIAL CUPS (DAYS 0–33)

The earlier pregnancy termination is attempted, the more likely it is to be safe and successful; however, no method has been shown to reliably terminate pregnancy before

day 5 after ovulation.[1,2] After the corpus luteum (CL) is fully functional (days 5–6 after ovulation), elective abortion is easily accomplished by causing luteolysis. The simplest method is intramuscular injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or a $PGF_{2\alpha}$ analogue.[1–4] The two products commonly used in mares, dinoprost tromethamine and cloprostenol, have similar efficacy, but the common side effects of sweating and mild colic are avoided with the administration of cloprostenol. Before maternal recognition of pregnancy (approximately by days 12–14) a single injection of either product (10 mg of dinoprost or 500 µg of cloprostenol) has been shown to cause lysis of the CL and effectively terminate pregnancy. After pregnancy recognition, two or more consecutive injections may be necessary to lyse diestrual, or secondary, corpora lutea.[3] Mares can be expected to return to estrus within 3 to 5 days.

Intrauterine infusion or lavage performed after day 6 also terminates pregnancy in mares. Abortion is likely caused by embryotoxic effects or release of endogenous $PGF_{2\alpha}$ as a result of cervical and uterine manipulations.[1] Sterile saline (2–3 L divided into 500- to 1000-ml aliquots) is the preferred solution for uterine lavage because it is relatively nonirritating, but infusions of Lugol's solution, dilute povidone-iodine, or nitrofurazone have been used successfully. These antiseptic solutions are potentially irritating to the genital tract and should be used with care. Chlorhexidine solution should not be used for intrauterine infusion.[5] Any technique that necessitates invasion of the cervix can result in bacterial contamination and endometritis.

Manual crushing of the conceptus can be performed easily between days 16 and 25 after ovulation. After day 25, this technique is more difficult and less efficacious. Transvaginal ultrasound-guided pregnancy reduction has been successful in terminating pregnancies between days 20 and 45.[6] Ovariectomy, although not a practical technique, consistently results in abortion during this period.[3]

INDUCED ABORTION WHILE ENDOMETRIAL CUPS ARE FUNCTIONAL (DAYS 34–120)

During days 34 to 120 of gestation, mares may not return to normal estrous cycles after pregnancy termination. Multiple doses of $PGF_{2\alpha}$ or an analogue have been shown to effectively terminate pregnancy in several studies.[5–7] Mares injected once or twice daily aborted 3 to 5 days after treatment.

Intrauterine infusion or lavage can also be employed to induce abortion, but it is more successful prior to chorioallantoic attachment to the endometrium (at approximately 80 days of gestation).[1,4] After attachment, manual invasion of the cervix, rupture of the chorioallantois, and manual extraction of the fetus and membranes are more expeditious.[4] A sterile saline lavage, following manual abortion, aids in removal of any remaining fetal membranes, blood clots, or fibrin tags.

Manual crushing of the conceptus after day 34 is technically difficult because of the size of the vesicle and the position of the uterus in some mares. Transvaginal ultrasound-guided allantocentesis, with aspiration of allantoic fluid, has successfully terminated pregnancy between days 40 and 65.[6,9,10]

INDUCED ABORTION AFTER 4 MONTHS OF GESTATION

Elective termination of pregnancy beyond the first trimester may be complicated by dystocia, retained placenta, and trauma to the genital tract. A variety of techniques are described in the literature, but there appears to be no consensus on which technique is the most efficacious.

Multiple injections of $PGF_{2\alpha}$ have resulted in abortions in mares between 100 and 245 days of gestation.[3,11] Abortion was induced using $PGF_{2\alpha}$ in two mares at 150 days' gestation; abortion occurred 37 hours after treatment in one mare and after 61 hours after treatment in the other.

A more reliable technique for the termination of late pregnancies is manual disruption of the fetal membranes and removal of the fetus. This is more easily accomplished if cervical dilation is enhanced by methods other than manual distention. Success has been reported with estrogen treatment (6–10 mg estradiol) 24 hours prior to induction of abortion.[4,11] Cervical dilation can also be achieved using intracervical application of PGE_2.[12] Oxytocin treatment (especially following estradiol therapy) may hasten the expulsion of the fetus and fetal membranes. Infusions of large volumes of saline have been recommended by many authors to aide myometrial contractions and expulsion of the fetal membranes.[1–4]

Transabdominal ultrasound-guided fetal cardiac puncture, followed by injection of potassium chloride, has been successful in reducing twin pregnancies in midgestation[13] and could be used for single pregnancy termination.

Intra-allantoic injection of dexamethasone (administered transcervically) induced abortion within 3 days in treated mares.[14] In contrast to other species, systemic dexamethasone (dose of 10 to 80 mg administered parenterally for 4 consecutive days) does not appear to cause abortion in mares.[15]

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CHAPTER 28

Surgical Correction of Abnormalities of the Female Reproductive Organs

JEAN-PIERRE HELD and JIM BLACKFORD

SURGICAL CORRECTIONS FOR ABNORMAL PERINEAL CONFORMATION

Types of Abnormal Conformations

Uterine contamination following a breakdown of the anatomic barriers that protect the uterine environment is a common cause of subfertility in mares. In normal mares, uterine contamination is prevented by three anatomic structures: vulvar lips, vulvovaginal sphincter, and cervix.

Vulvar Lips

Ideally, over 80% of the labia should lie in a vertical plane below the ischiadic arch of the pelvis. The Caslick index (La, where L = vulvar length and a = the angle of declination of the vulvar lips) has been developed to enable objective evaluation of the need for corrective surgery.[1] Mares with an index above 100 may benefit from surgery and those with an index above 150 should definitely show improved fertility following surgery (Fig. 28-1).

Vulvovaginal Sphincter

The tightest seal is formed at the junction of the vestibule and the caudal vagina, near the urethral opening. Integrity of the seal depends on the paravaginal connective tissue and a thin muscle layer.

Cervix

Infertility may result due to loss of cervical integrity, with infections manifested as a secondary complication. The cervix represents the last protective barrier between the uterus and the caudal portion of the tubular reproductive tract. A physiologic loss of integrity occurs during estrus, when the cervix dilates under the effects of estrogen.

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Cervix

Infertility may result due to loss of cervical integrity, with infections manifested as a secondary complication. The cervix represents the last protective barrier between the uterus and the caudal portion of the tubular reproductive tract. A physiologic loss of integrity occurs during estrus, when the cervix dilates under the effects of estrogen.

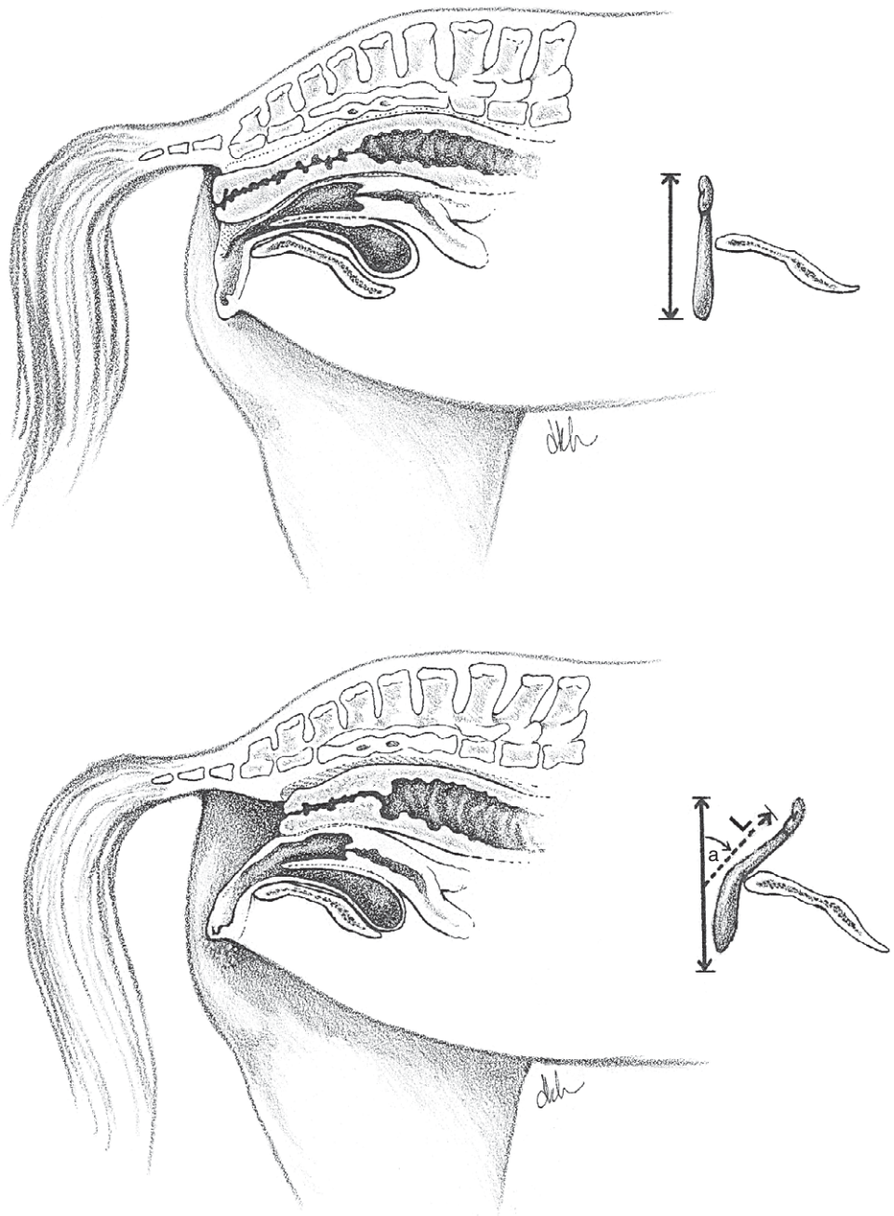

Fig. 28-1 Caslick index determination. *Upper,* normal conformation. *Lower,* abnormal conformation.

Etiology of Poor Perineal Conformation

Dorsal displacement of the vulvar lips in relation to the ischiadic arch predisposes to pneumovagina, particularly in multiparous mares. With each pregnancy, the labia are pulled further dorsocranial, predisposing to aspiration of air and fecal contaminants into the vagina. Effective vulvar length also increases with age. *Poor body condition* reduces the amount of paravaginal fat and paravaginal muscle tone, drawing the anus cranially, thus predisposing to windsucking. *Dystocia* may stretch the perineal body, thereby disrupting integrity of the cranial vaginal seal. Perineal lacerations that disrupt the integrity of the vaginal wall and paravaginal tissue increase the risk of pneumovagina. Multiparous mares with poor vulvovaginal sphincter tone may aspirate air in the absence of a recessed anus, particularly during estrus. *Cribbing* mares with marginally normal perineal conformation may need episioplasty because negative pressure created in the abdominal cavity during cribbing pulls the anal sphincter cranially, predisposing to windsucking.

Surgical Procedures

Episioplasty (Caslick's Surgery)

Episioplasty[2] is the surgical procedure used to reduce the length of the vulvar opening. Following is a summary of the steps involved:

1. Determine the amount of vulvar closure required: A finger should be introduced into the dorsal commissure of the vulva and moved ventrally until the ischiadic arch is palpated. The vulvar lips should be closed to a point 1 to 2 cm ventral to that point, leaving a 3- to 4-cm opening.
2. Anesthesia of the vulvar edges: A 22-gauge needle is introduced along the planned incision line at the mucocutaneous junction of the labia. Lidocaine or Carbocaine is deposited while the needle is withdrawn. Approximately 5 to 10 ml of anesthetic solution is needed on each side. Special attention should be given to adequately block the tissue proximal to the dorsal commissure.
3. Excision of vulvar lip edges: After placing traction on the vulvar lips in a ventral direction, scissors are used to remove a narrow strip of tissue at the mucocutaneous junction. No more than 0.5 cm of tissue should be removed (Fig. 28-2, *A*).
4. Suture the cut edges: Absorbable suture material should be used to avoid the need for suture removal. A continuous or interrupted suture pattern can be used to appose the tissues (Fig. 28-2, *B*).
5. Tetanus prophylaxis should be administered in nonimmunized mares. In instances in which a mare is to be mated after undergoing an episioplasty, a breeding stitch may be indicated. This is a simple interrupted suture of 0.6 mm polypropylene loosely placed 0.5 to 1 cm dorsal to the ventral edge of the sutured lips. Its purpose is to prevent separation of the vulvar labia during intromission. If the breeding stitch is placed too far ventrally, it may cause abrasions to the stallion's penis (Fig. 28-2, *C*).

Episiotomy

Owners should be instructed to have the episioplasty opened approximately 1 month prior to foaling, at the time the mare is vaccinated and dewormed. Opening of the episioplasty is accomplished by incising the sutured vulvar lips up to the dorsal commissure. A local anesthetic may be necessary.

Perineoplasty

In mares that have a very loose vulvovaginal sphincter along with a cranially displaced vulva, episioplasty may not prevent pneumovagina. For these mares, perineoplasty may be performed by resecting a portion of the dorsal vaginal wall and apposing the denuded tissue, resulting in narrowing of the caudal vagina.[3]

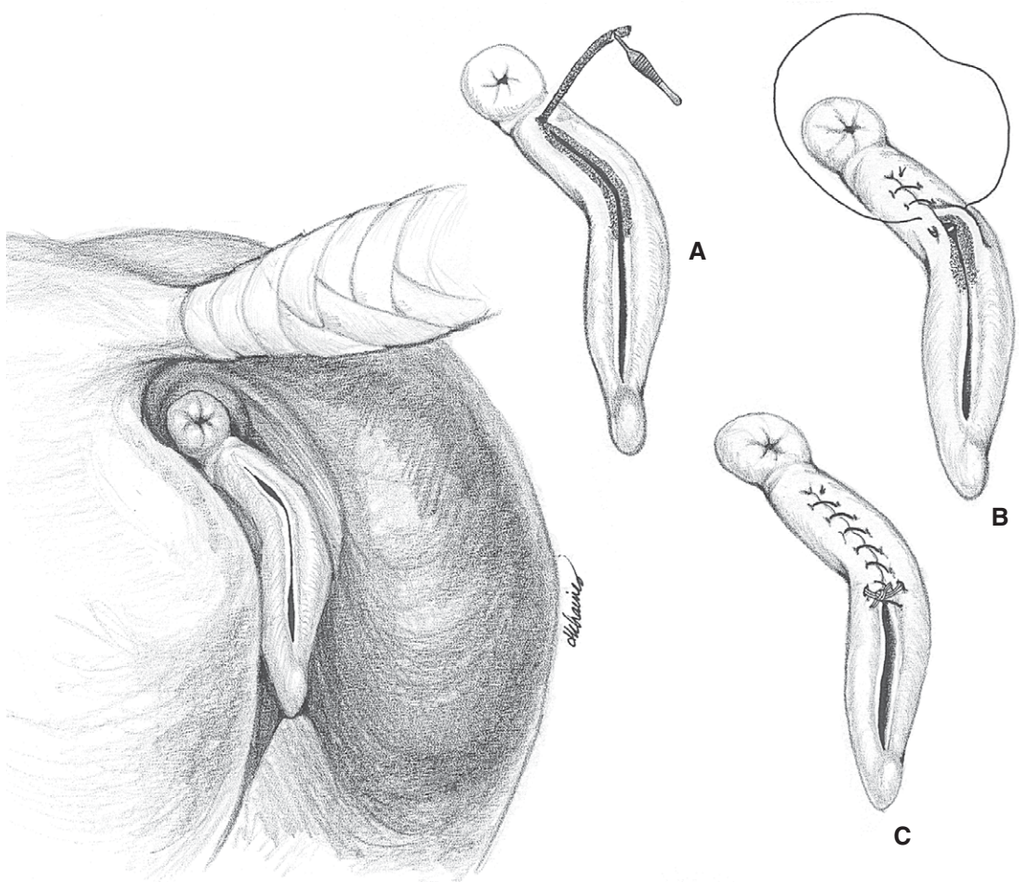

Fig. 28-2 Episioplasty.

1. Analgesia: Epidural anesthesia is preferred over local infiltration due to the large surgical area.
2. Exposure of the surgical site: Adequate exposure may be achieved with stay sutures or towel clamps placed at the 10, 12, and 2 o'clock positions of the vulvar opening.
3. Tissue resection: A triangle-shaped section of mucosa is resected from the dorsal vaginal wall. The base of the triangle is at the mucocutaneous junction of the vulvar lips, at the 10 and 2 o'clock positions. The apex lies on the dorsal midline, above the urethral opening (Fig. 28-3, *A*).
4. Perineal sutures: After the towel clamps are removed, evenly spaced horizontal mattress sutures (No. 1 or 2 polypropylene) are used to appose the edges of the vaginal mucosa, narrowing the caudal vagina. To achieve even tension along the suture line and prevent tissue necrosis, rubber tubing can be used under the exposed suture loops on the perineum (Fig. 28-3, *B*). The perineal and vulvar skin is closed with a No. 0 polypropylene suture in a simple continuous pattern (Fig. 28-3, *C*).

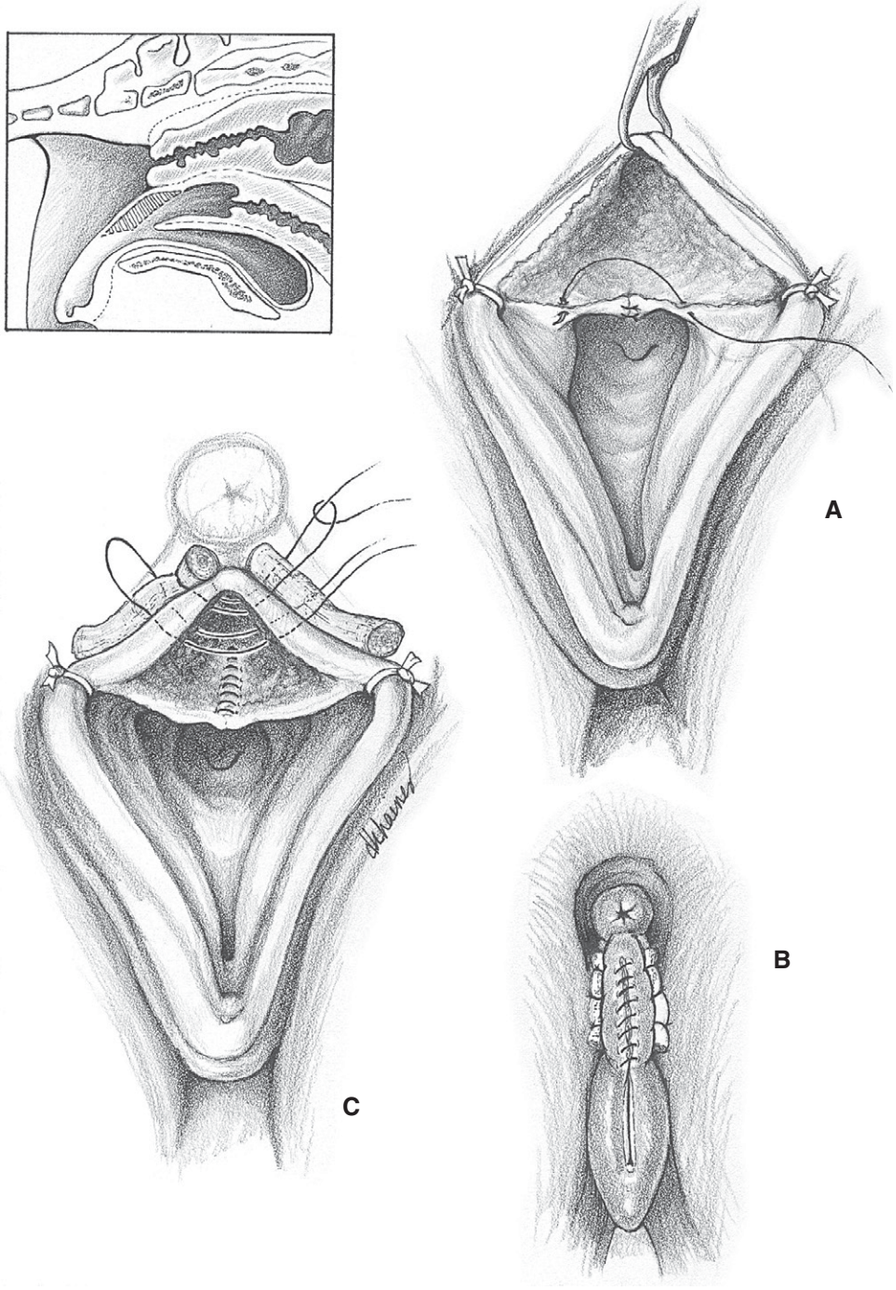

Fig. 28-3 Perineoplasty.

5. Suture removal: All sutures should be removed 10 to 14 days after surgery.

SURGICAL CORRECTION OF URINE POOLING IN THE MARE

Urine pooling (vesicovaginal reflux) is a well-known cause of infertility. It is commonly encountered in multiparous mares because of relaxation of the ovarian and uterine ligaments. Other predisposing factors for urine pooling are weight loss, abnormal perineal conformation (recessed anus), and the combination of edema and relaxation of the reproductive tract that occurs during estrus or the early postpartum period. Mares that experience urine pooling due to poor perineal conformation should have Caslick's surgery. Mares may pool urine only during estrus and suspect mares should be examined during standing heat. Several techniques have been described for surgical correction of the condition. The goal is to extend the existing urethra caudally. Three surgical approaches are described here; however, surgical corrections often fail postoperatively and must be repeated.

Presurgical Preparation

The mare is restrained in stocks, with or without tranquilization. Analgesia is achieved with epidural anesthesia. The rectum is emptied, and the tail is wrapped and tied out of the way with a rope attached around the mare's neck. The vagina is cleaned with sterile lactated Ringer's solution.

Surgical Procedures

Posterior Fixation of Transverse Fold

This technique is among the first described to extend the urethra.[4] The urethral fold is pulled caudally, and its edges trimmed and sutured to incisions in the vaginal wall with No. 0 absorbable material. In most cases, caudal traction on the fold creates excessive tension and results in failure of the suture line. Additionally, it is difficult to achieve good apposition between the edges of freshened urethral fold and the vaginal wall (Fig. 28-4).

Urethral Extension (Shires Technique)[5]

After placement of a 30 French Foley catheter in the urethra (Fig. 28-5, *A*), interrupted horizontal mattress sutures (No. 0 synthetic absorbable material) are placed in the vaginal mucosa, sparing the catheter. As the sutures are tightened, folds of mucosa close over the catheter. Adequate mucosa must be present on the formed crest to allow mucosal tissue to be trimmed. Following excision, a simple continuous suture pattern is used to appose the cut edges. After completion of the procedure, the catheter is removed (Fig. 28-5, *B* to *F*).

Urethral Extension (McKinnon Technique)

With this technique, the urethra is extended using vaginal mucosa. In contrast to other techniques, the denuded tissue present after forming the tunnel is not oversewn but is allowed to heal by second intention (Fig. 28-6, *A* to *D*).[6]

Postoperative Management

When a urethral catheter has been placed, it should be removed upon completion of the surgery because its presence may lead to excessive straining by the mare. Catheters tend to become rapidly obstructed by urinary crystals. Postoperative treatment should include administration of procaine penicillin G (20,000 IU/kg) for 5 days and tetanus prophylaxis. Mares should not be bred for at least 3 weeks. Thereafter, breeding management should include artificial insemination or hand breeding to prevent injury to the extended urethral fold during intromission.

PERINEAL LACERATION REPAIR IN THE MARE

Perineal lacerations associated with parturition represent a significant cause of infertility in mares. Lacerations are most common in primiparous mares, and the injury appears to be more prevalent in mares than in other species. This may be because of the rapid onset and completion of parturition in the horse. Perineal lacerations are divided into four categories:

First degree: Tear of the vulvar lips only.
Second degree: Tear of the perineal body including the connective tissue and paravaginal muscle at the level of the vulvovaginal sphincter.
Third degree: Cloaca formation from a tear extending through the rectovaginal shelf, perineal body, anal sphincter, and vulvar lips.
Rectovaginal fistula: Communication between rectum and vagina without disruption of the perineal body, anal sphincter, or vulvar lips.

Repair of First-Degree Lacerations

Vulvar lip lacerations may occur from insufficient opening of an episioplasty before foaling or overstretching during foaling. When these lacerations are limited to the dorsal commissure, adequate repair is usually attained with an episioplasty to prevent windsucking and pneumovagina.

Repair of Second-Degree Lacerations

Tears involving the perineal body, including the vulvovaginal sphincter, may predispose to urine pooling, pneumovagina or both. These tears require surgical repair of both the perineal body and vulvar lips. In addition, urethral extension may be required to correct pooling of urine in the cranial vagina.

Third-Degree Lacerations and Rectovaginal Fistulae

Presurgical Management

Immediate repair of third-degree lacerations is not recommended. Edema and contaminated and devitalized tissue make immediate repair difficult, if not impossible. The initial injury may require 3 to 6 weeks to heal. Mares

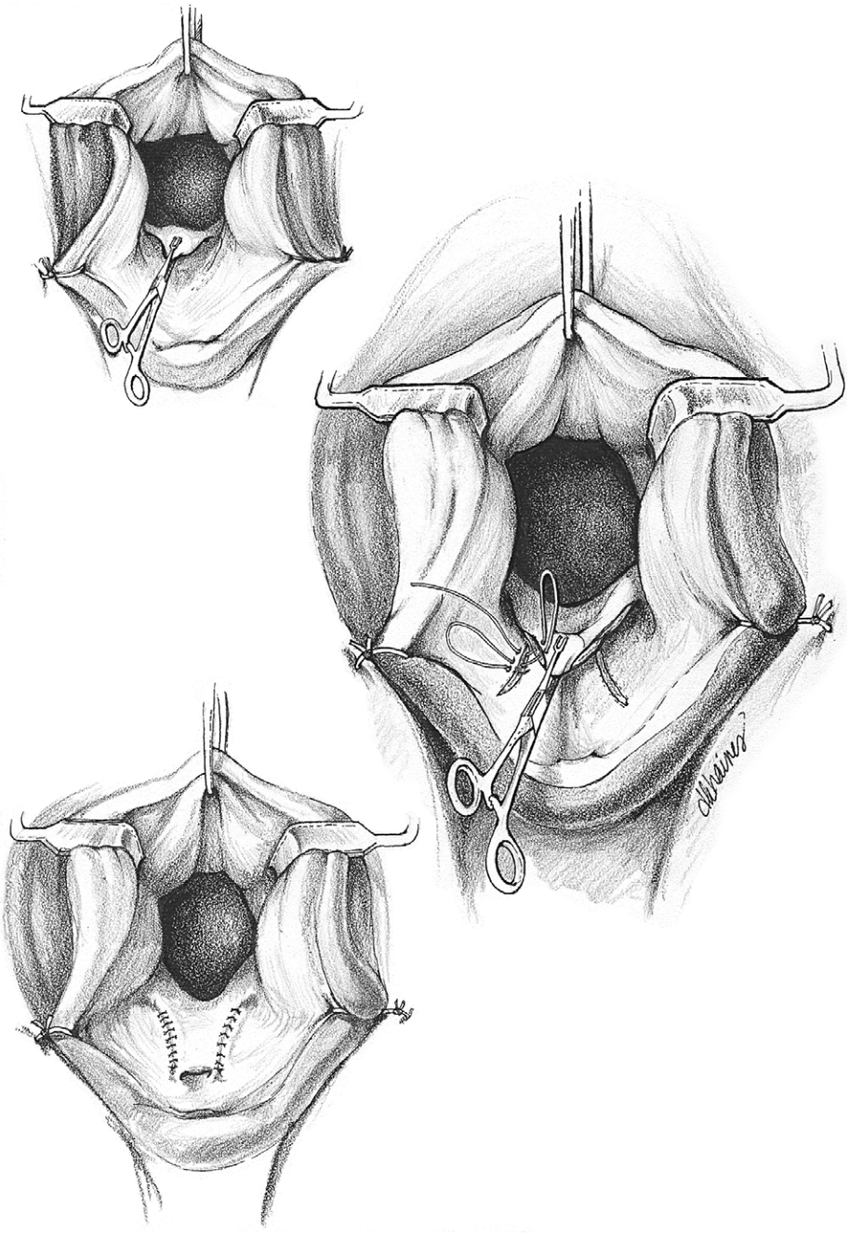

Fig. 28-4 Posterior fixation of transverse fold.

that deliver a dead foal may be repaired at that time. In the case of delivery of a live foal, it is recommended to delay repair until the foal is weaned. Presurgical preparation is critical for a successful surgical repair.

Presurgical Dietary Management

Soft to pasty fecal consistency is critical to prevent suture dehiscence. Firm to normal fecal consistency places excessive tension on the suture lines and predisposes to dehiscence. Fluid feces increase the risk of introducing contaminants into the suture line, predisposing to rectovaginal fistulation. Ideally, feces should have a consistency similar to that of cattle, with no formed fecal balls. Several methods have been used to control fecal consistency. A lush pasture usually keeps feces soft but the quantity passed is high. When alfalfa pellets or complete feed pellets are fed, they should be soaked in hot water for approximately 6 hours, and mineral oil (2–4 L per feeding) should be mixed in the mash (if the mare tolerates it) or given by nasogastric tube. Some mares with very dry feces may respond favorably to magnesium sulfate (Epsom salt) or sodium sulfate (Glauber's salt). The latter is more palatable and, therefore, 100 to 200 g/day can be added to the mash. The use of cathartics is not recommended as they may cause fluid feces. During the 1- to 2-week period prior to surgery, bedding should be

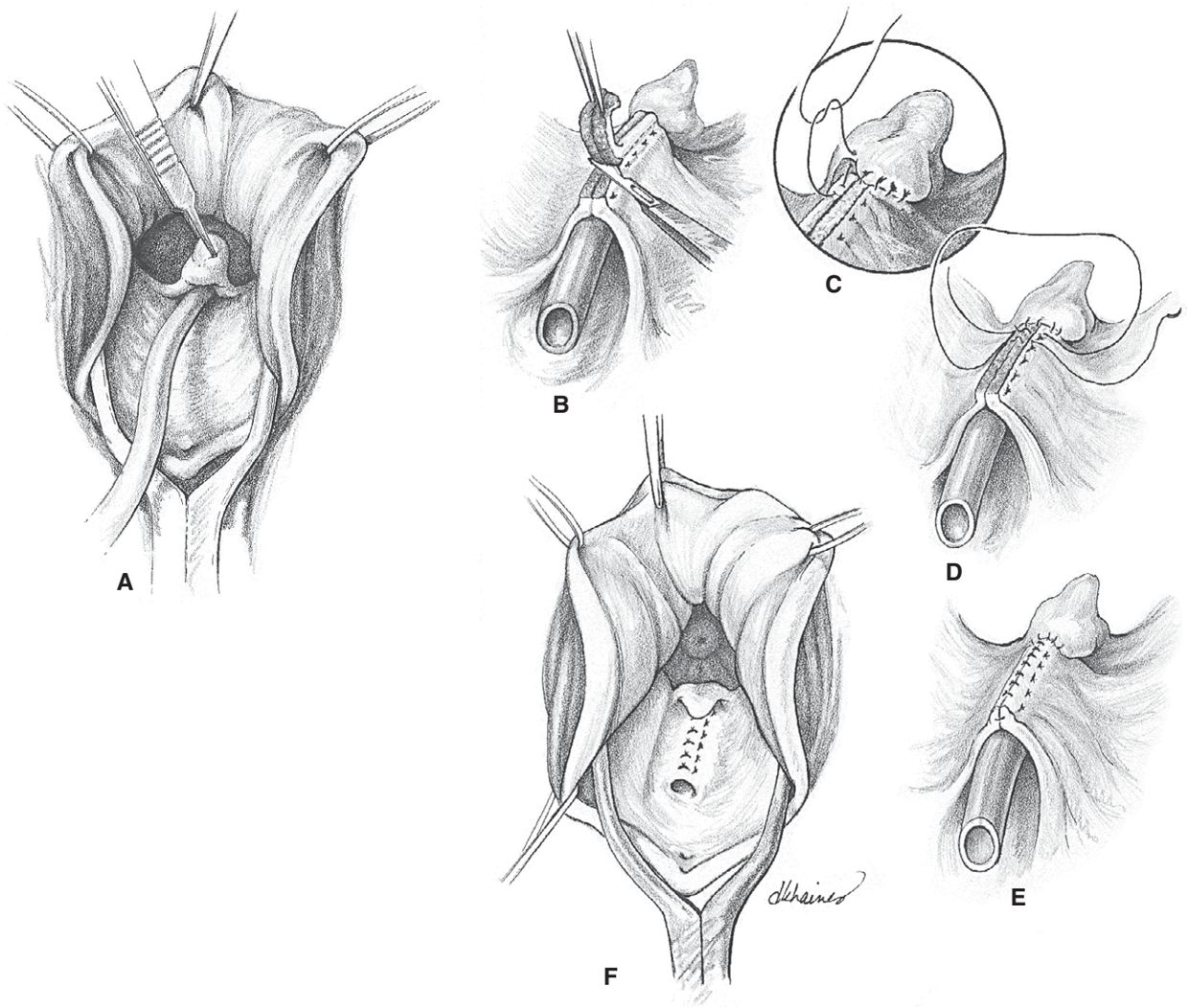

Fig. 28-5 **A,** Urinary catheter placement. **B** to **F,** Urethral extension (Shires technique).

avoided. Withholding feed for 2 days preceding surgery will reduce the chance of fecal contamination during surgery.

Repair of Third-Degree Lacerations

Mare preparation. The mare is placed in a stock, and epidural anesthesia is administered. The use of xylazine for epidural anesthesia is preferred because tone in the anal sphincter remains unaffected by this compound. Acepromazine maleate may be beneficial in fractious mares. Intravenous xylazine should be avoided because of its diuretic effect. The wrapped tail can either be tied overhead or pulled to the side and anchored around the patient's neck. Fecal material is manually removed from the rectum, and the perineal area is thoroughly scrubbed.

First-stage repair. Adequate exposure of the surgery site is achieved by vulvar retraction with stay sutures, towel clamps, or modified Finochietto retractors (Aanes Retractors, Scanlan Instruments, Englewood, Col.). Visualization is improved with a head lamp.

The initial surgery reconstructs the rectovaginal shelf. A No. 15 blade is used to incise and separate the rectovaginal shelf approximately 2 to 3 cm cranial to the defect (Fig. 28-7, *A*). This incision is continued caudally on each side of the common vault, following the margin between the rectal and vaginal mucosa. Scar tissue should not be removed, as tissue loss increases tension on the suture line. Dissection should continue through the mucosa, submucosa, and connective tissue. Care must be taken not to injure the deep (and large) paravaginal vessels.

The rectovaginal shelf is reconstructed using a modified Goetze[7] or Aanes[8] technique. A continuous horizontal mattress or simple continuous pattern is used to close the vaginal mucosa (Fig. 28-7, *B*). An interrupted 6-bite pattern is used for the rectal shelf (Fig. 28-7, *C*). Care must be taken to prevent placement of sutures through the rectal mucosa. The simple continuous vaginal suture can be either sutured first or alternated with the suture approximating the rectal shelf. The suture material used

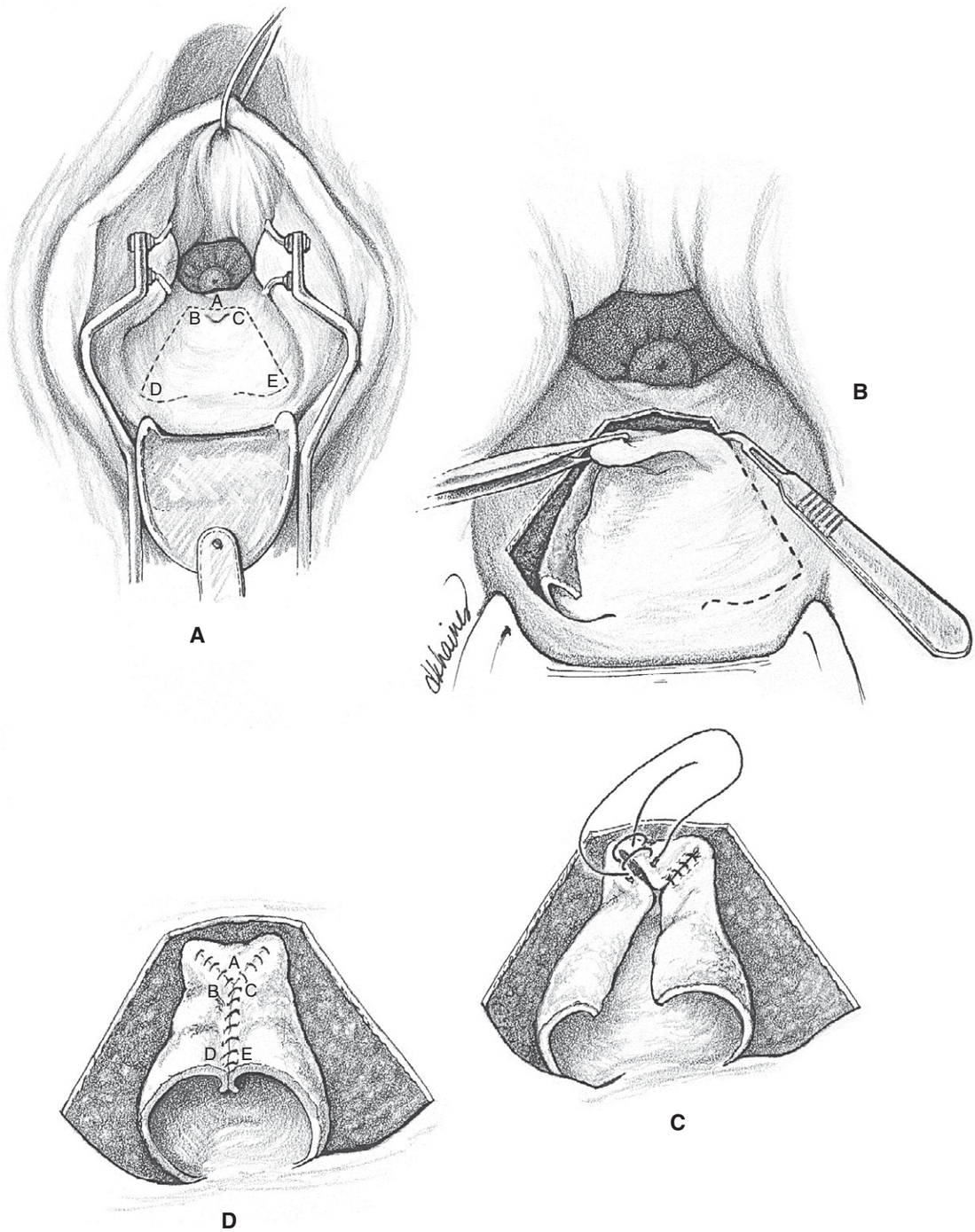

Fig. 28-6 Urethral extension (McKinnon technique).

can be either No. 1 polyglactin (Vicryl, Ethicon, Somerville, NJ) or polydioxanone (PDS, Ethicon, Somerville, NJ) for rectal shelf reconstruction and No. 0 polyglactin for the continuous vaginal suture. The perineal body is not repaired during the initial surgery so as to decrease tension on the sutures when the mare defecates. Diet management as described previously is of prime importance in the postoperative phase because excessive tension placed on the sutures may result in dehiscence. A broad-spectrum antibiotic should be given for 4 to 5 days and appropriate prophylaxis for tetanus is administered as indicated.

Second-stage repair. Three to 4 weeks following reconstruction of the rectal shelf, the perineal body is

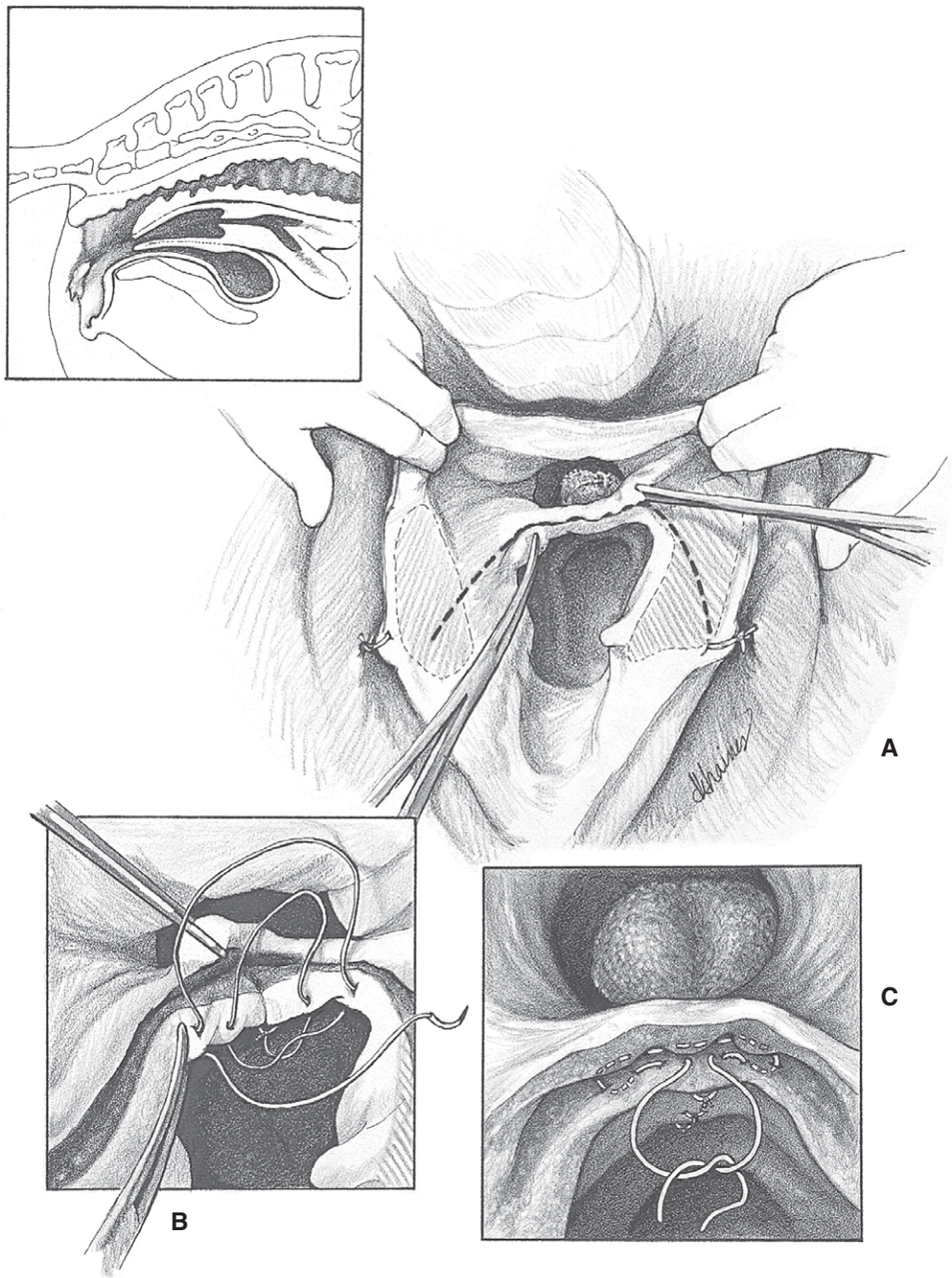

Fig. 28-7 Perineal laceration: first-stage repair.

repaired. The mare is prepared for surgery as described for the first stage. The procedure consists of removing the epithelium on the ventral aspect of the torn anal sphincter. The dorsal vulvar lips are trimmed as for Caslick's surgery. Deep sutures of No. 1 polyglactin or polydioxanone are placed to reconstruct the perineal body and anal sphincter. Special attention must be given to the amount of anal closure achieved. The skin of the vulva and the anus may be closed with either absorbable or nonabsorbable suture material.

Postoperative care. It is advisable to maintain the mare on the laxative diet described earlier for an additional 10 to 14 days.

Alternative Technique for Repair of Third-Degree Lacerations

In mares with substantial loss of perineal tissue, the operative technique described previously can result in excessive tension and predispose to dehiscence.

Surgical technique. The mare is prepared as described previously. A No. 15 blade is used to incise and separate the rectovaginal shelf. The rectal shelf is reconstructed with a simple continuous or interrupted Lembert pattern using No. 1 polyglactin or polydioxanone suture. The vaginal shelf is repaired with a simple continuous or continuous horizontal mattress pattern. The rectovaginal space is left exposed to granulate and heal by second intention. The perineal body and anal sphincter can be reconstructed as described previously. If wound dehiscence occurs, additional surgery is usually easier because of the increased amount of tissue present after healing.

Repair of Rectovaginal Fistulae

The traditional way to repair rectovaginal fistulae is to convert them to third-degree lacerations and repair them as described previously.[9] Retraction of the vulva is accomplished as previously described. The edges of the fistula (vaginal side) are prepared for closure as depicted in Figure 28-8. An interrupted vertical mattress or Lembert pattern is used for the deep layer of sutures that appose the rectal mucosa. An interrupted or continuous horizontal mattress pattern is used to close the vaginal mucosa. The suture material should be either No. 1 polyglactin or polydioxanone. Sufficient vaginal mucosa should be removed around the fistula to provide adequate tissue apposition when the everting rectal sutures are placed. The rectal mucosa should not be penetrated. Postoperative care is similar to that described for third-degree laceration repair. An alternate surgical method is to position the mare in dorsal recumbency under general anesthesia.[10]

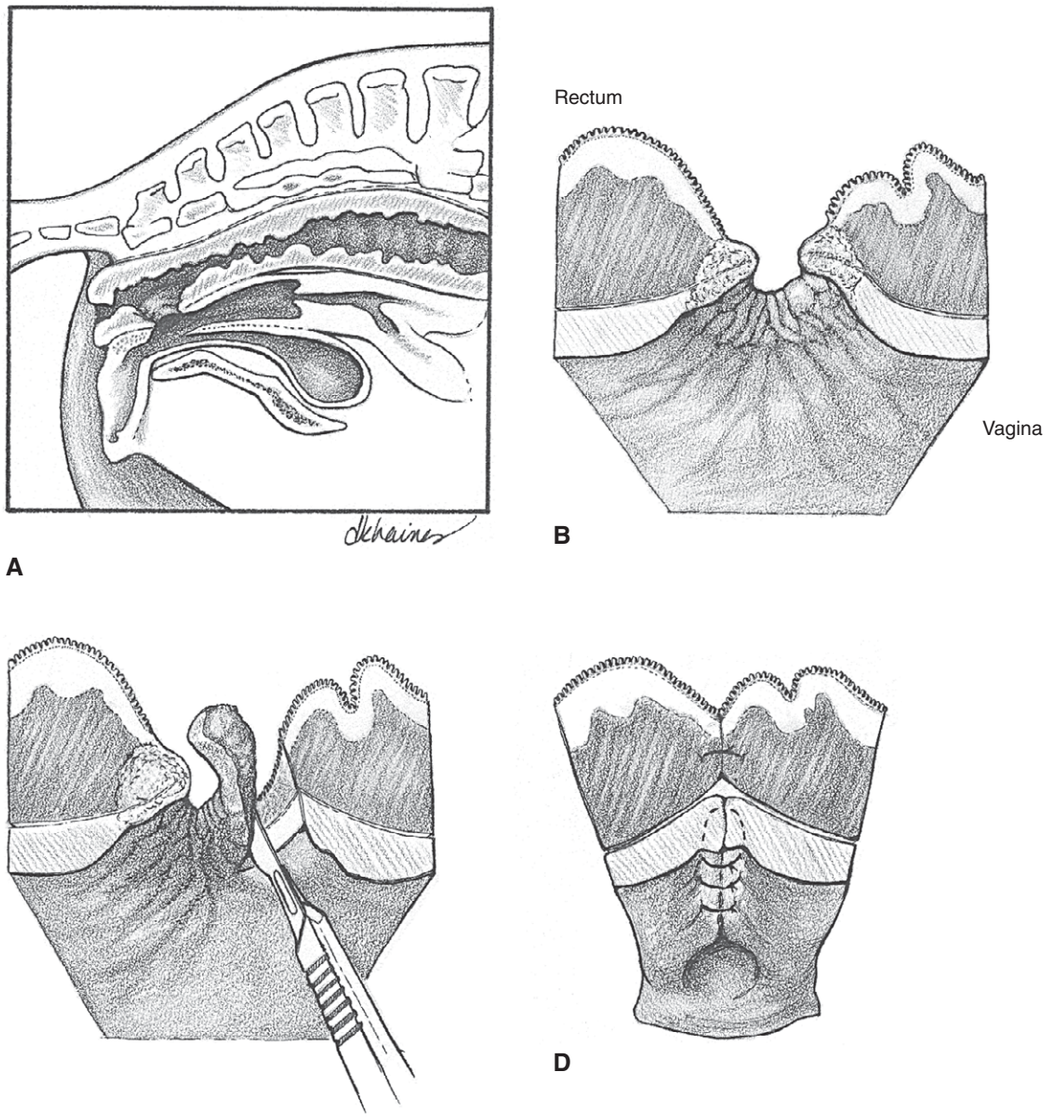

Fig. 28-8 Rectovaginal fistula repair.

The most important factors for successful repair of rectovaginal fistulae and third-degree lacerations are as follows:

1. Assessment of tissue available to build the rectovaginal shelf
2. Avoidance of removal of any tissue during shelf preparation
3. Avoidance of excessive tension on suture line
4. Building a shelf of adequate thickness
5. Adequate fecal consistency

OVARIECTOMY

Two common indications for ovariectomy in mares are neoplasms, such as granulosa cell tumors, and the desire for permanent control of objectionable behavior during estrus in performance horses. The three surgical approaches available are through the ventral midline, the flank, and the vaginal wall. Selection of the most appropriate approach depends on ovarian size.

Ventral Midline Approach

When ovaries are greater than 15 cm in diameter, a ventral midline approach is preferred because of the limited space available in the flank to exteriorize the ovary. Heavy, large tumors are generally easier to exteriorize through a ventral midline approach, as the weight of the mass stretches the ovarian pedicle.

Flank Approach

This technique is appropriate for unilateral removal of ovaries up to 15 cm in diameter. The combination of thick abdominal musculature and the small space between the last rib and the tuber coxae makes flank removal of large ovaries difficult. Care is taken to avoid the cecum if a right flank approach is used.

Preoperative Management

Food should be withheld from the mare for 24 to 36 hours to reduce distention of the gastrointestinal tract. The surgical site is clipped and shaved in a routine manner. The ventral border of the shaved area should allow extension of the incision if needed.[11]

Analgesia

The mare is restrained in stocks and sedated with detomidine hydrochloride (20 µg/kg IV). Analgesia of the surgical site is achieved using the inverted L block technique with 2% lidocaine solution.

Surgical Technique

A vertical or slightly oblique incision is started at a point centered between the tuber coxae and the last rib (Fig. 28-9). For ovaries greater than 10 cm in diameter or in mares with a small paralumbar fossa, the incision is initiated 10 to 15 cm ventrally, as in a low flank approach. The external oblique, internal oblique, and transverse abdominal muscles are manually separated from the center of the incision outwardly (grid pattern). With

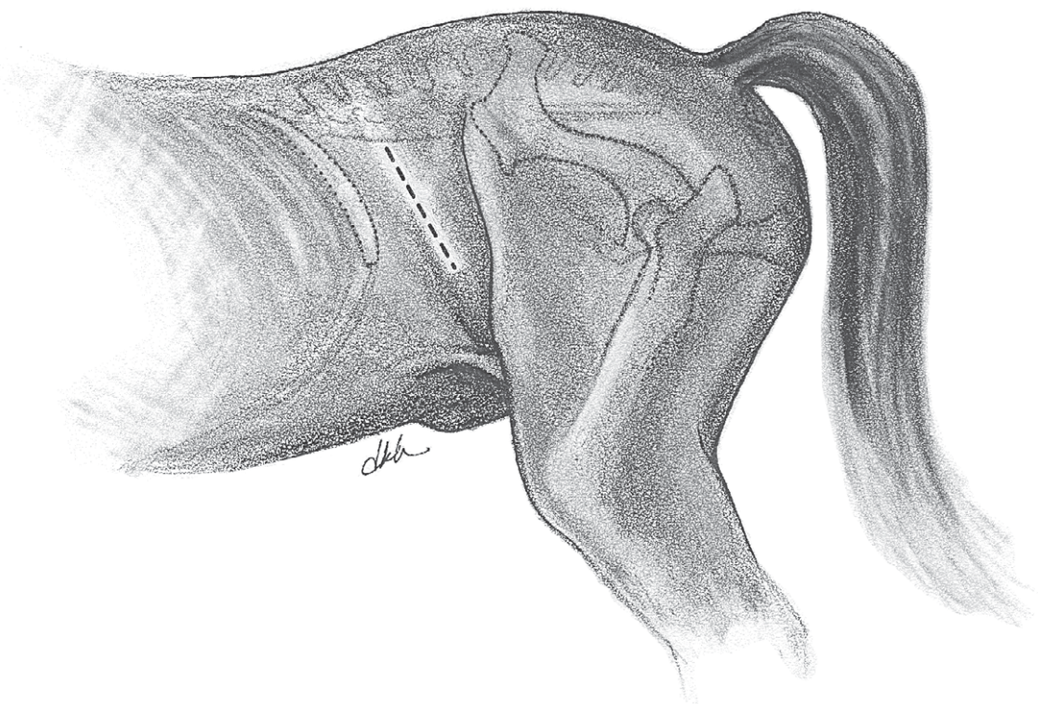

Fig. 28-9 Ovariectomy: flank approach.

larger ovaries (10–15 cm), part or all of these muscles may have to be transected. Before the abdomen is entered, the peritoneum is sprayed with 5 to 10 ml of lidocaine. This decreases the mare's discomfort when the peritoneum is penetrated. Blunt Mayo dissecting scissors are used to perforate the peritoneum, and the opening is enlarged manually. A gloved hand is extended in a caudal direction to locate the ovary. If it cannot be located, the uterus is palpated at the pelvic brim and the uterine horn followed laterally until the ovary is isolated. A lidocaine-soaked gauze sponge attached to umbilical tape (to prevent accidental loss of the sponge in the abdomen) is wrapped around the ovarian pedicle to desensitize it. This procedure is important to minimize pain that may cause the mare to collapse. The surgeon exteriorizes the ovary by placing fingers behind the ovary and pushing it through the incision. If this method fails, an assistant palpating transrectally can push the ovary toward the abdominal incision, enabling the surgeon to place one or two nylon stay sutures into the tunica albuginea of the ovary. The combination of rectally pushing the ovary toward the abdominal opening, and pulling on the nylon sutures helps in the exteriorization of larger ovaries. To prevent postoperative hemorrhage, enlarged vessels should be individually ligated and the ovarian pedicle transfixed with No. 2 polyglactin, avoiding injury to the larger blood vessels. This is particularly important for congested blood vessels associated with granulosa cell tumors. As the pedicle is transected, the stump should be observed for hemorrhage. When the ovarian ligament is short and does not allow sufficient exposure to ligate the vessels, a chain écraseur (Fig. 28-10) can be used as described later, or surgery can be postponed until the ovarian ligament is elongated enough under the tumor's weight to allow proper exteriorization. Alternatively, an automatic stapling device (TA-90, United States Surgical, Norwalk, Ct.) can be used to place two rows of stainless steel staples around the ovarian pedicle. Care must be taken to avoid inclusion of omentum or intestine during the stapling process and to evenly distribute the tissue in the stapling jaws.[12] If the tissue is too thick, the staples will not close properly. Individual muscle layers are apposed with a simple continuous pattern using No. 0 polyglactin or polydioxanone. The fascia of the external abdominal oblique muscle is carefully closed using an interrupted or simple continuous pattern of No. 1 polyglactin or polydioxanone. The skin is closed with nonabsorbable material. Because seromas at the surgery site are a frequent occurrence, a Penrose drain can be inserted under the skin sutures.

Postoperative Care

The use of a broad-spectrum antibiotic such as trimethoprim/sulfonamide for 4 days, and flunixin meglumine (Banamine, Schering Corporation, Kenilworth, NJ) for visceral pain is recommended. The Penrose drain should be removed 3 days postoperatively and the skin sutures removed 10 days postoperatively. The mare should be kept in a stall for 48 hours and observed for any signs of shock or depression that could indicate hemorrhage from the ovarian stump.

Colpotomy Approach

A colpotomy is the surgical approach of choice for bilateral ovariectomy of normal mares. To minimize the risk of postoperative hemorrhage, surgery is performed during seasonal anestrus. If surgery is done during the breeding season, the mare should be in diestrus.

Preoperative Care

Food is withheld from the mare for at least 24 hours. The rectum is evacuated, as a full rectum can be easily penetrated when the vaginal incision is made. The perineum is surgically prepared and the vagina flushed with 1% povidone-iodine solution (Betadine, Purdue Frederick Co, Norwalk, Ct.).

Analgesia

The mare is sedated and epidural anesthesia is performed to paralyze the tail and prevent straining during surgery. Pronounced analgesia is important because any unexpected reaction from the mare during the vaginal incision may result in injury to the rectum or iliac artery.

Surgical Technique

The vaginal lips are spread, which allows the vagina to dilate with air and facilitates the vaginal incision. Incision of the vaginal fornix is usually made at the 2 o'clock position, 2 to 4 cm dorsolateral to the cervix. This position is preferable to the 10 o'clock location because it provides more direct access to the right ovary, which is located further cranially. The incision is made with a No. 15 Bard-Parker blade (Becton-Dickinson, Rutherford, NJ) protected by the thumb and fingers, limiting forward thrust. Only the vaginal mucosa should be incised in

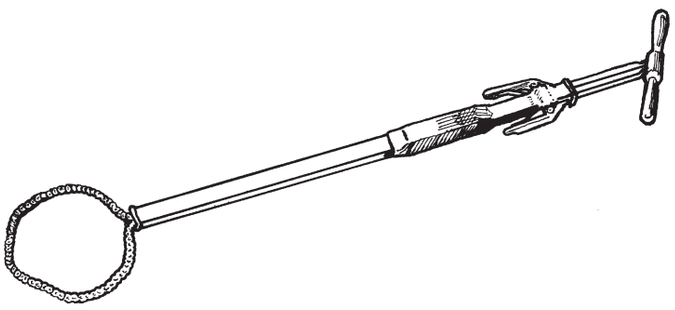

Fig. 28-10 Chain écraseur.

order to minimize trauma to the iliac artery and the rectum. Penetration of the abdomen is done with blunt dissecting scissors; the opening is then manually enlarged. Excessive pain is prevented by slowly stretching the peritoneal opening. After the ovary is located, ovarian ligament analgesia is performed as previously described. An écraseur (see Fig. 28-10) is introduced into the abdomen with the chain slightly slack, allowing one finger through the loop. The ovary is identified and the chain looped around the base of the ovary. Edges of the chain should be carefully examined for trapped intestine or mesentery after initial tightening but before crushing and cutting of tissue. An assistant then gradually tightens the chain while the surgeon holds the ovary. To prevent excessive pain and provide for optimal crushing, the chain is tightened one click every 10 to 15 seconds until the stump is transected. The instrument and the ovary are then removed. During the procedure it is important to keep the instrument as far cranial as possible, preventing tension on the ovarian ligament.

Postoperative Care

Antibiotics and flunixin meglumine are administered as previously described. The mare should be confined to a stall with daily exercise (hand walking), for 5 to 7 days. After that time, the combination of swollen tissues and wound contraction makes herniation of abdominal organs into the vagina unlikely.

CERVICAL LACERATION REPAIR[13]

Cervical lacerations occasionally occur, primarily during dystocias in older, multiparous mares or with inappropriate fetotomy techniques. Less commonly, these tears can occur during normal parturition. Examination of mares with suspected cervical tears should be made during diestrus. The variability in cervical relaxation present during estrus makes a determination of the extent of cervical muscle disruption difficult to determine. However, this relaxation is advantageous for the surgical procedure.

Diagnosis

A gloved hand is used to vaginally palpate the cervix. The location, extent of the tear, and amount of scar tissue that should be removed is accurately determined. Caudal retraction of the cervix for surgery will distort the tissue and make such an evaluation difficult. Defects of the external os of the cervix or tears not affecting over one third of its length generally do not require surgical repair.

Preoperative care and analgesia may be done as described for ovariectomy, colpotomy approach.

Surgical Repair

Surgical repair is best performed during estrus, because of the easier access to the body of the cervix. The vulvar lips are retracted and secured to the perineum with towel clamps or stay sutures. The cervix is retracted caudally using three No. 3 nylon or umbilical tape stay sutures placed deep into the external os or cervical body. The suture ends are left long enough to allow for retraction by helper(s). The use of instruments with long handles, similar to the ones used in third-degree perineal laceration repair is essential. A full-thickness incision is made starting 2 cm cranial to the defect and extending caudally to the external os. Excessive scar tissue is removed and the cervix closed in a two- or three-layer closure. In the three-layer closure, a No. 0 resorbable monofilament suture material such as PDS is used to close the cervical musculature in a simple interrupted or continuous suture pattern. The cervical and vaginal mucosa are closed with a No. 2-0 PDS suture in a simple continuous pattern.

Postoperative Care

Postoperative care should include antibiotic and anti-inflammatory medications for 3 to 4 days. The use of progesterone (300 mg IM daily) for 10 to 14 days may increase cervical tone more rapidly. The mare should be rechecked 30 days following surgery. If healing is adequate, she may be inseminated at that time. Natural breeding should be avoided for 6 months to prevent further cervical injury.

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CHAPTER 29

Embryo Transfer and Newer Assisted Reproductive Techniques for Horses

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Embryo transfer has been the most widely utilized assisted reproductive technique for mares; it has been used to (1) obtain foals from performance mares, (2) obtain multiple foals from individual mares each year, (3) obtain foals from 2-year-old mares, (4) obtain foals from reproductively unsound mares, and (5) obtain foals from mares with nonreproductive health problems. In addition, embryo transfer has served as an important research tool for studying early pregnancy in the mare. Although embryo transfer was initially proposed as a promising method for obtaining foals from aged, subfertile mares, experiments utilizing oocyte transfer[1] and embryo transfer[2] have documented that many oocytes/embryos produced by aged, subfertile mares are inherently defective and have low survival rates after transfer to recipient mares; therefore, aged, subfertile mares are not optimal candidates for embryo transfer.

Although embryo transfer provides a means of obtaining pregnancies from some mares that might not otherwise be capable of producing offspring, some mares cannot provide embryos for transfer. Mares in which embryo transfer may not be successful include those with (1) ovulatory failure, (2) chronic endometritis, or (3) anatomic problems (e.g., cervical adhesions). However, these mares could be used as oocyte donors and continue to produce foals through newer assisted reproductive techniques such as oocyte transfer, in vitro fertilization, and intracytoplasmic sperm injection. In addition, stallions with low sperm numbers or poor semen quality could also benefit from some of these newer technologies. This chapter will review current embryo transfer procedures and provide an overview of the current status of several newer assisted reproductive techniques for horses.

EMBRYO TRANSFER

The first successful equine embryo transfer was reported in 1972[3]; however, it was not until the early 1980s that embryo transfer became an accepted clinical procedure in the equine breeding industry. At that time, widespread utilization of embryo transfer was limited by the need to maintain recipient mares at the site of embryo collection, or ship donor mares to a centralized embryo transfer facility. In the late 1980s, a technique for cooling equine embryos was identified[4] and led to the development of a practical method of short-term (≤24 hours) storage and transportation of equine embryos. That development allowed embryos to be collected in the "field" and then shipped to a centralized facility for transfer to suitable recipient mares. The ability to transport cooled embryos provided veterinarians with the opportunity to offer embryo transfer service without the onerous task of maintaining recipient mares and eliminated the need to ship donor mares to a centralized facility.

Mare Management

Donor Mare

If indicated, a complete breeding soundness examination of the donor mare should be performed to assess her suitability for use in an embryo transfer program. If abnormalities are identified that warrant treatment (e.g., bacterial endometritis), appropriate therapy should be completed before embryo transfer procedures are performed. Breeding management of the donor involves teasing to monitor reproductive behavior; use of transrectal palpation and ultrasonography to monitor ovarian follicular activity and ovulation; and use of exogenous hormones to synchronize estrus and ovulation. When in heat, the donor is examined daily to monitor follicular growth, which allows optimal timing of insemination with fresh, cooled, or frozen semen. The day of ovulation is detected is designated as Day 0. Historically, a practical and efficacious means of superovulating mares has not been available; however, a commercially available formulation of equine follicle stimulating hormone (eFSH) has recently become available. Mares treated with eFSH had an average of 3.9 ovulations and 1.9 embryos recovered during a treatment cycle compared to 1.0 ovulation and a 0.5 embryo recovery rate for control mares.[5] Work is continuing to develop an optimal eFSH treatment protocol for superovulation in mares.

Recipient Mares

Proper selection and management of recipient mares may be the most important factor affecting the success of an embryo transfer program. Recipient mares should have normal estrous cycles, and must be free of uterine or ovarian abnormalities. The optimal age of recipient mares is 3 to 10 years. Synchronizing estrus between donor and recipient mares can be accomplished with routine protocols using prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) alone or in

combination with exogenous progesterone.[6] When in heat, recipients are examined daily with transrectal palpation and ultrasonography to monitor follicular growth and detect ovulation. The "window" of synchrony between ovulation in recipient and donor mares is +1 to –3 days (i.e., the recipient mare can ovulate 1 day before to 3 days after the donor mare).[7] In an effort to eliminate the need for synchronizing recipient and donor mares, progestin-treated, ovariectomized mares have been used as embryo recipients[8–11]; however, the success with their use has been variable, and the practice has not been widely adopted.

Embryo Recovery

Equine embryos are selectively transported through the oviduct into the uterus between days 5 and 6 after ovulation,[12] at which time they are at the compact morula to early blastocyst stage of development. After entering the uterine lumen, the size of the embryo increases dramatically as it develops into an expanded blastocyst (Table 29-1).[13] Although embryos can be recovered on days 6 to 9 (Table 29-2),[14–20] the optimal time of embryo collection is day 7 or 8; currently, our preference is to perform embryo recovery on day 8. The primary indication for recovering embryos on day 6 is for freezing embryos.[7] Embryos are not routinely collected on day 9, because their transfer success rate is generally lower than day 7 or 8 embryos.[7]

Embryo collection is performed using transcervical uterine lavage (Fig. 29-1). We currently use an 80-cm silicone balloon-tipped catheter (VEUF-80, Bivona, Inc., Gary, IN 46406) with an inside diameter of 8.0 mm (French size 33); however, other styles of flushing catheters are available. After placing the catheter, the uterus is flushed three to four times with warm (30° to 35° C) flush medium. Historically, the most widely used flush medium has been Dulbecco's phosphate buffered saline (DPBS) containing 1% (v/v) fetal or newborn calf serum, penicillin (100 units/mI), and streptomycin (100 μg/ml); however, more recently, many practitioners have begun to use a zwitterion-buffered flush medium containing antibiotics and bovine serum albumin (emCare Complete Flush Solution, Professional Embryo Transfer Supply, Inc., Canton, TX 75103-0188) or polyvinyl alcohol (ViGro Complete Flush Solution, AB Technology, Pullman, WA 99163).

Table **29-1**

Diameter of Equine Embryos Recovered From the Uterine Lumen

Day Postovulation	Number of Embryos	EMBRYO DIAMETER (mm) Mean	Range
6	121	0.208	0.132–0.756
7	144	0.406	0.136–1.460
8	142	1.132	0.120–3.980
9	41	2.220	0.730–4.520

Adapted from Squires EL, Cook VM, Voss JL: Collection and transfer of equine embryos. Bull 1, Colorado State University, Animal Reproduction Laboratory, Fort Collins, Col, 1985.

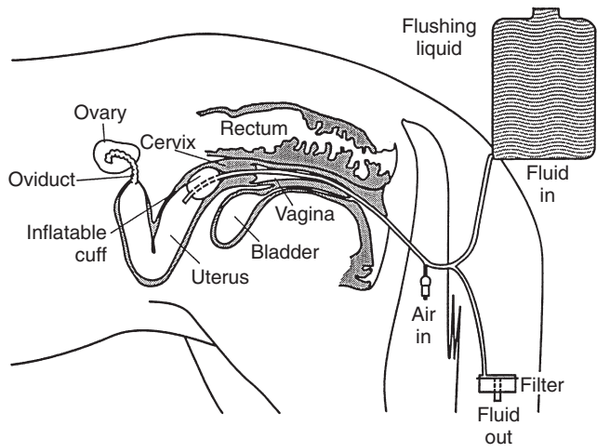

Fig. 29-1 Diagram of the flushing system for recovering uterine embryos.

Table **29-2**

Effect of Day Postovulation On Equine Embryo Recovery Rate

Reference	DAY 6 (%)	7 (%)	8 (%)	9 (%)
Iuliano et al.[14]	21/32 (66)	68/90 (76)	50/61 (82)	—
Castleberry et al.[15]	3/13 (23)	15/22 (68)	4/8 (50)	—
Squires et al.[16]	86/137 (63)	73/96 (76)	218/293 (74)	43/53 (81)
Meira et al.[17]	70/127 (55)	23/41 (56)	—	—
Wade and Gallagher[18]	—	26/45 (58)	31/47 (66)	—
Bowen et al.[19]	12/23 (52)	—	25/31 (81)	—
Fleury and Alvarenga[20]	—	106/215 (49)	388/669 (58)	18/33 (55)
Total	192/332 (58)	311/509 (61)	716/1109 (65)	61/86 (71)

Regardless of which medium is used, using gravity flow, the uterus is filled with 1 to 2 L of medium during each flush (4 to 8 L used during entire procedure). After filling the uterus, the fluid is allowed to flow back out through the catheter and is passed through a 0.75-μ embryo filter. It is important that the embryo filter not overflow or run dry; filters are available that are designed to prevent both from occurring. The fluid passing through the filter is collected to monitor its recovery. After the first flush, the uterus is massaged per rectum during subsequent flushes, which may aid suspension of the embryo(s) in the medium and enhance fluid recovery. The majority (>90%) of fluid infused into the uterus should be recovered, and should be free of cellular debris or blood. Recovery of cloudy fluid indicates the mare had an active endometritis at the time of the embryo recovery and warrants further diagnostic evaluation. When present, blood contamination is often associated with vigorous massage of the uterus or manipulation of the catheter.

At the completion of the flush, the filter cup is emptied into a sterile search dish with grid and the filter is rinsed with approximately 50 ml of flush medium. The fluid is then examined for the embryo(s) using a stereomicroscope at approximately 15× magnification. Larger embryos (e.g., day 8) are generally visible with the naked eye. When an embryo is identified, it is washed by transferring it sequentially through several (3 to 10) 1-ml drops of "holding medium," which consists of an enriched formulation of flush medium; after washing, the embryo is placed into a small petri dish containing the same medium. The embryo is then examined at high magnification (40 to 80×) and graded on a scale of 1 (excellent) to 4 (poor).[21] Embryos can be handled using a 0.25- or 0.5-ml semen-freezing straw, 25-μl glass capillary pipette, or other suitable instrument attached to an appropriate syringe. Whenever an embryo is drawn into a handling instrument, the medium containing the embryo should be surrounded on each side by an air bubble and blank medium. This prevents the embryo from accidentally being pulled out of the instrument should the tip touch something absorbent.

Once embryos are placed into the holding medium, they should either be expeditiously processed and packaged for transport (or frozen) or be transferred into an appropriate recipient mare.[22] While awaiting packaging for transport or immediate transfer to a recipient, equine embryos appear to be quite tolerant of temperatures between room temperature (25° C) and body temperature (37° C). However, efforts should be made to prevent rapid or extreme changes in temperature.

Packaging Embryos for Transport

Since the late 1980s, equine embryos have been cooled and transported using methods developed by Carnevale and associates,[4] which utilizes Ham's F-10 nutrient mixture as the holding/cooling medium. Prior to use, Ham's F-10 medium must be buffered by diffusing a mixture of 90% N_2, 5% O_2, and 5% CO_2 gas through the medium for 3 to 5 minutes, after which it is supplemented with 10% (v/v) fetal or newborn calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). Because Ham's F-10 medium must be "gassed" prior to use, it requires having an appropriate compressed gas cylinder and regulator. In addition, once gassed, Ham's F-10 has a limited shelf-life; therefore, many practitioners choose to have the embryo transfer facility that will receive the embryo provide Ham's F-10 just before the embryo collection as part of an embryo shipping kit. More recently, equine embryos have been successfully cooled and transported using the complete medium holding solutions described above,[22] which eliminates the need to prepare the special Ham's F-10 solution.

To package an embryo, the transport medium is filter-sterilized into "snap-cap" tube, leaving a small air gap at the top of the tube. The embryo is then carefully transferred into the medium, the cap is securely snapped onto the tube, and the tube is wrapped with Parafilm. A 50-ml centrifuge tube is then filled with transport medium (unfiltered), and the 5-ml tube containing the embryo is placed into the 50-ml centrifuge tube. The cap of the 50-ml centrifuge tube is closed, eliminating as much air as possible, and it is wrapped with parafilm. The packaged embryo is then placed into an Equitainer, which passively cools the embryo to 5° C. Under those conditions, embryos can remain viable for at least 24 hours, during which time they can be transported via commercial airline or priority overnight delivery to the embryo transfer facility.

Freezing Embryos

The primary advantage of freezing embryos is that it obviates the need to have a synchronized recipient mare available at the time of embryo collection, since the embryo can be frozen and then transferred at a later date when a recipient mare is available. In addition, it would facilitate the international movement of equine embryos. Although "conventional" embryo freezing procedures using glycerol or other cryoprotective agents have been reasonably successful for equine embryos, to do so requires expensive and specialized equipment, as well as approximately 90 minutes to complete the freezing procedure. In addition, after thawing, embryos are generally moved through a series of solutions to dilute the cryoprotectant and other components of the freezing medium before transfer, which makes the post-thaw process laborious. In contrast to conventional freezing procedures, vitrification is a process that utilizes high concentrations of cryoprotectants that can be completed rapidly (<15 min) without specialized equipment using a commercially available vitrification kit.[23] After thawing, vitrified embryos can be directly transferred from the straw into the recipient mare, since further dilution or handling of the embryo is not necessary.

To vitrify an embryo, after "washing" the embryo with a standard embryo holding medium, the embryo is moved sequentially through three separate vitrification solutions (VS1, VS2, and VS3), and then it is loaded into a 0.25 ml polyvinyl chloride straw.[23] The embryo is held in VS1 for 5 minutes and then transferred to VS2 for an additional 5 minutes, after which it is transferred to VS3.

The embryo should remain in VS3 for less than 1 minute, which includes the time to load the embryo into the straw and initiate freezing. The embryo is loaded into the straw in approximately 30 ul of VS3; the embryo (in VS3) is "sandwiched" in the straw between an air bubble on either side and approximately 90 ul of a 0.5 M galactose solution beyond that (on both sides of the embryo). The galactose solution will be mixed with the embryo in VS3 *after* thawing. Once the straw is loaded, the open end is sealed, and the straw is placed into liquid nitrogen vapor for 1 minute before it is plunged into the liquid nitrogen, after which it is moved to a storage tank. A straw is thawed by holding it in room temperature air for 10 seconds, and then it is placed into a room temperature (20° to 22° C) water bath for an additional 10 seconds, after which the contents of the straw are mixed by "flicking" it five to seven times (like a thermometer). The straw is then allowed to lay flat for 6 to 8 minutes at room temperature, after which the tip of the straw is opened and then loaded into a standard transfer gun for transfer into a suitable recipient mare. Using this vitrification technique, Carnevale et al.[23] reported a 62% (16/26) pregnancy rate at day 16 after transfer of embryos. However, like conventional freezing procedures, only small (<300 micron) embryos tolerate vitrification procedures suitably. Therefore, embryos that will be vitrified should be collected on day 6 to very early on day 7.

Surgical and Nonsurgical Embryo Transfer Procedures

Regardless of whether embryos are transferred immediately after recovery; cooled and transported; or frozen prior to transfer, the transfer procedure can be performed surgically or nonsurgically. Historically, surgical transfer has provided the highest pregnancy rates and most consistent results, generally resulting in pregnancy rates of approximately 70% to 75% 1 week after transfer[24]; however, recent reports of nonsurgical transfer have demonstrated the success rates can be equal to, or greater than, those obtained with surgical transfer.[20,25]

At this time, nonsurgical has become the standard method of transferring equine embryos and has generally been performed using (1) a standard artificial insemination pipette, (2) a disposable plastic "insemination gun," or (3) a reusable stainless steel insemination gun. Regardless of which instrument is used, an outer guard is generally placed over the transfer instrument. When performing a nonsurgical transfer, the recipient mare is placed in stocks and sedated, and the perineal area is cleaned and prepared using standard procedures. The operator places a sterile plastic sleeve over his or her arm, and a sterile surgeon's glove is placed over the plastic sleeve. A small amount of sterile lubricant is placed on the back of the operator's hand and applied to the vulva. The tip of the transfer instrument (covered by the outer guard) is placed in the palm of the hand and protected by placing the operator's thumb over the tip. The instrument is introduced into the vagina, and the tip of the outer guard is introduced approximately 0.5 cm into the external cervical os, at which point the instrument is advanced through the outer guard and passed through the cervical canal into the uterine body. The embryo can be deposited in the uterine body or in one of the uterine horns; to deposit the embryo in the uterine horn, the tip of the instrument is guided into the horn using transrectal manipulation. Currently, there is no evidence that the site of embryo placement (uterine body versus horn) during nonsurgical transfer influences the outcome. Once the transfer instrument is positioned appropriately, the embryo is expelled as the transfer instrument is withdrawn slightly, so the tip is not pushed up against the endometrium as the embryo is deposited into the uterus.

NEWER ASSISTED REPRODUCTIVE TECHNIQUES

The successful application of new assisted reproductive procedures requires that equine gametes are collected and handled appropriately. For successful fertilization, the oocyte must be viable and at the correct stage of maturation (meiotic and cytoplasmic). Oocytes "resting" in the ovary are in prophase of meiosis I and contain a distinct nucleus (germinal vesicle). During in vivo maturation, which occurs during the 36 hours prior to ovulation, the nuclear membrane breaks down, and meiosis resumes and progresses to metaphase II. At ovulation, the oocyte has reached, and is arrested in, metaphase II,[26] the stage at which it is fertilizable. Metaphase II oocytes are characterized by the presence of the first polar body.

For commercial application of these new assisted technologies, oocytes are primarily collected by transvaginal ultrasound-guided follicle aspiration (TVA). Oocytes can be collected from mature preovulatory follicles or from immature follicles. Oocytes collected from small, immature follicles must undergo maturation in vitro. The development of successful culture systems for routine in vitro maturation of equine oocytes is currently an area of considerable research interest.

Oocyte Transfer

Oocyte transfer involves collection and surgical transfer of a donor mare's mature oocyte into a recipient mare's oviduct so that fertilization and subsequent embryonic development occur within the reproductive tract of the recipient. This procedure has been referred to as gamete intrafallopian transfer (GIFT), which implies that both gametes (oocyte and spermatozoa) are transferred into the oviduct; however, as it is currently being performed in the mare, this procedure involves only the transfer of an oocyte, for the recipient mare is inseminated with semen from the desired stallion using standard insemination procedures. Because the recipient mare is inseminated, her oocyte must be removed to prevent it from being fertilized.

Oocyte transfer does not require ovulation to occur in the donor and completely bypasses all of the tubular genitalia of the donor mare; therefore, it may be especially well suited for donor mares with ovulatory failure and those with chronic problems associated with the tubular genitalia (pyometra, etc.). In addition, oocyte transfer has been proposed as an alternative method of obtaining pregnancies from any mare in which embryo transfer

has been unsuccessful, regardless of the underlying reason.

Although the first equine oocyte transfer resulting in the birth of a foal was reported in 1988,[27] only recently has the procedure been performed as a clinical procedure. In an initial report, Carnevale and associates[28] described their results using oocyte transfer in a commercial breeding program involving 38 mares (mean age 21 years) with reproductive problems. Donor mares consisted of those with a wide range of reproductive abnormalities including persistent endometritis, pyometra, cervical fibrosis, repeated development of anovulatory hemorrhagic follicles, and idiopathic reproductive failure. Oocytes were collected from donor mares using transvaginal ultrasound-guided follicle aspiration, which was well tolerated by the donor mares without any complications. Oocyte collection rates were high, with oocytes recovered during 80% of cycles. Oocyte collection was performed approximately 24 hours after administration of human chorionic gonadotropin or the gonadotropin-releasing hormone agonist delorelin acetate in an effort to recover oocytes that were in the process of nuclear and cytoplasmic maturation. At the time oocytes were collected, cumulus cell expansion was evaluated to assess the degree of oocyte maturation; oocytes with a fully expanded cumulus were transferred as soon as possible into a recipient mare's oviduct, while oocytes with less cumulus expansion were incubated in culture medium for 12 to 26 hours prior to transfer. Of a total of 90 oocytes recovered from these donor mares during 99 cycles, 75 were transferred into 64 recipient mares, which resulted in 20 mares becoming pregnant (31%).

Carnevale and associates[28] noted that the success of oocyte transfer in their study was affected by oocyte and semen quality. The most important factor affecting oocyte quality was mare age. Reduced fertility in mares at or over 20 years old has been associated with poor oocyte quality; embryo development rates were significantly reduced when oocytes from aged mares, compared to young mares, were transferred into young recipient mares.[1] In addition, morphologic evaluation of oocytes from aged and young mares using light and electron microscopy demonstrated that although some oocytes from aged mares are morphologically similar to oocytes from young mares, more oocytes from aged mares had morphologic anomalies such as large vacuoles and oblong shapes.[29] Because semen quality also affected the success of oocyte transfer, the authors recommend the use of stallions with high-quality semen to maximize the success of the procedure.

In a subsequent report, Carnevale and associates[30] further described the various factors that affect the clinical success of oocyte transfer. Like the initial report,[28] their experience clearly demonstrates that oocyte transfer can be used to obtain pregnancies from mares with a wide range of reproductive problems that otherwise would probably not be able to produce offspring.

In Vitro Fertilization

In vitro fertilization (IVF) has been used successfully for many years in the treatment of human infertility, and techniques are now available for producing large numbers of IVF embryos from several domestic species. Although the first foal produced by IVF was reported by Palmer and associates in 1991,[31] a repeatable method for successfully performing IVF in horses has not been developed. The primary biologic barrier to IVF appears to be an inability to effectively capacitate equine spermatozoa in vitro, a process that is necessary in order for spermatozoa to be capable of fertilizing an oocyte. Because of the lack of progress in this area, recent research has focused on alternatives to traditional methods of IVF.

Intracytoplasmic Sperm Injection

Intracytoplasmic sperm injection (ICSI) is one of the modified forms of IVF being developed for use in horses that involves using a micromanipulator to inject a single spermatozoon into the cytoplasm of a mature metaphase II oocyte. By mechanically fertilizing an oocyte by placing a spermatozoon into the oocyte, ICSI eliminates the need for the spermatozoon to bind to and penetrate the zona pellucida and oocyte plasma membrane, which appear to be the aspects of spermatozoon-oocyte interaction that fail to proceed appropriately during standard IVF procedures.

The first foal produced using ICSI was reported in 1996,[32] and since that time there have been further reports of the success of the ICSI procedure.[33,34] In a recent report,[35] Hinrichs reviewed the current status of the ICSI procedure, focusing heavily on their extensive work in that area. Currently, the blastocyst development rate is 23% to 44% following ICSI and in vitro embryo culture, with a 50% pregnancy rate after transfer of those in vitro–produced embryos to surrogate mares. Unfortunately, approximately 50% of the embryos that establish pregnancy in recipient mares are lost during early gestation, which currently limits the overall efficiency of the procedure. Although ICSI must still be considered an experimental procedure, it holds great potential for assisted reproduction in the mare. In addition, because ICSI requires only a single spermatozoon, it has tremendous potential application for stallion management (e.g., subfertile stallions, frozen semen).

Sexed Semen

There is considerable interest in developing methods of separating X- and Y-chromosome bearing spermatozoa, to produce "sexed" semen that when inseminated allows gender selection of the resulting offspring; insemination of X-bearing spermatozoa will result in female offspring, and insemination of Y-bearing spermatozoa will result in male offspring. The first foal (a filly) conceived with "sexed" semen was born during the summer of 1998 (E.L. Squires, personal communication).

Semen is "sexed" using a flow cytometer, an instrument that physically separates X- and Y-bearing spermatozoa; however, using current instrumentation, only 1000 to 1500 viable, sexed spermatozoa can be sorted per second.[36] Although that appears to be an extremely fast rate of sorting, at that rate, it would require 4 to 5 days of continuous operation to produce enough "sexed"

spermatozoa for one insemination dose using the standard number of spermatozoa required for conventional insemination of fresh semen (i.e., 500 million progressively motile spermatozoa). That limitation has been responsible for stimulating research to develop new insemination methods that are successful when a limited number of spermatozoa are used. In addition to use with sexed semen, low-dose insemination techniques may be beneficial when inseminating thawed frozen semen of low quality or limited quantity, or when inseminating semen from subfertile stallions.

Three methods of "low-dose" insemination have been investigated: (1) surgical insemination into the oviduct,[37] (2) transcervical endoscopic insemination at the uterotubal junction or into the oviduct,[38–40] and (3) deep intrauterine insemination near the uterotubal junction.[41] Although surgical oviductal insemination can achieve pregnancies with extremely low numbers of spermatozoa (50,000 to 150,000), it has limited practical application at this time. Compared to oviductal insemination, endoscopic insemination is more practical and can achieve pregnancies with a slightly higher number of spermatozoa (~1 million), but requires expensive equipment that is not well suited to field use. Currently, deep intrauterine insemination is the most practical method of low-dose insemination because it can be performed in the field with readily available equipment, but it requires more spermatozoa (5 to 25 million) than oviductal or endoscopic insemination. Continued research is needed to further refine low-dose insemination techniques before "sexed" semen can be widely utilized.

Nuclear Transfer (Cloning)

When Dolly the sheep was cloned using somatic cell nuclear transfer,[42] it was recognized as a major scientific milestone because it conclusively demonstrated that a fully differentiated somatic cell can be genetically "reprogrammed" back to the undifferentiated state of a one-cell zygote (embryo), which can then initiate and undergo complete embryonic/fetal development resulting in the birth of an animal that is genetically identical to the original cell donor. Although the specific methods used for cloning using nuclear transfer can differ, it is generally performed by micro-manipulating and fusing two cells. One cell, referred to as the nuclear donor or "karyoplast," is derived from the animal to be cloned; typically, donor cells are maintained in tissue culture, from which one cell is selected for each nuclear transfer procedure. The other cell, referred to as a "cytoplast," is a mature unfertilized oocyte from which the genetic material (polar body and metaphase plate) has been removed. The cytoplast contains numerous cellular factors (e.g., mRNA, proteins, etc.) that play an important role in the "reprogramming" of the genetic material (i.e., genes) of the donor cell that enables the cloned embryo to initiate the complex sequence of events leading to embryonic and fetal development. The reconstituted embryo uses the donor cell DNA as the template for subsequent gene expression, which results in a genetic clone of the donor animal.

Equine cloning was first successful in 2003, when we reported the live birth of three mule foals cloned from a fetal fibroblast cell line[43] and Galli et al.[44] reported the live birth of a horse foal cloned from an adult fibroblast cell line. Since then, several more cloned horses have been produced.[35,45] Despite these successes, like other species, the current efficiency of equine cloning is very low, as evidenced by the fact that only 0.7%[35] to 2.7%[43] of reconstructed cloned equine embryos result in the birth of live offspring. In addition, similar to other species, there is evidence the individual cell donor can influence the success of equine cloning.[44,46] Clearly, further work is needed to increase the efficiency of equine cloning.

For the equine industry, potential uses of equine cloning include (1) preservation of genetics from individual animals that would otherwise not be able to reproduce, such as geldings, (2) preservation of genetic material of endangered and/or exotic species such as the Mongolian Wild Horse (Przewalski's horse), and (3) because of the companion animal role that horses fill for some individuals, it is likely that some horse owners will have individual animals cloned for emotional fulfillment. Of these, cloning geldings to produce intact males for breeding purposes has been the first direct application of equine cloning.[45] Although some breed associations (e.g., The Jockey Club, American Quarter Horse Association) do not currently allow the registration of cloned animals, for some equine sporting activities (dressage, show-jumping, etc.) breed registry status is irrelevant, which eliminates that regulatory impediment to the utilization of cloning technology.

Veterinarians can assist their clientele prepare for cloning of individual animals (if so desired) by "banking" tissue from those animals. There are currently several commercial companies that will isolate and store cells from tissue samples collected from animals for subsequent cloning purposes. These companies typically provide the veterinarian with a tissue collection and transport kit; the procedure involves aseptically collecting a small skin biopsy, which is placed in tissue culture medium and returned to the company, where cells are grown in tissue culture. Once the cells have grown in culture, they can be used immediately for cloning or they can be harvested and stored frozen in liquid nitrogen for use in the future. Ideally, tissue should be collected from a live animal; however, in an emergency it may be possible to collect a suitable sample post mortem. To maximize the likelihood of recovering viable cells, tissue from the deceased animal should be kept refrigerated between and 3° and 8°C (37° and 47°F); the chance of recovering viable cells diminishes when the tissue has been frozen or stored at temperatures above 10°C (50°F).

SUMMARY

Embryo transfer is a valuable assisted reproductive technique in the mare. Although the use of embryo transfer in commercial breeding programs was initially hampered by the need to provide suitable recipient mares at the site of embryo collection, or transport donor mares to a centralized embryo transfer facility, the development of methods for successfully transporting cooled embryos eliminates the need of maintaining recipients on site. That, coupled with the fact that the materials necessary

for embryo collection and transport are well suited to a field setting, more veterinarians can now provide embryo transfer service to their clientele who wish to utilize this technology.

For mares in which embryo transfer cannot be performed successfully, newer assisted reproductive techniques such as oocyte transfer, IVF, or ICSI may provide a means through which offspring can be obtained. In addition, sexed semen and nuclear transfer procedures will provide horse owners with new options for the reproductive management of their animals. Although these new technologies are just beginning to have clinical application at this time (primarily oocyte transfer), with continued development, they will offer new alternatives to our approach of managing subfertile and infertile mares and stallions.

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CHAPTER 30

Clinical Reproductive Physiology and Endocrinology of Bulls

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The bull exerts major influences on herd fertility and production whether he is bred with many females, using assisted reproductive technologies, or with relatively few via natural service. Despite this, relatively little selection pressure for reproductive traits has been placed on most bull populations. Furthermore, multiple-sire breeding, as practiced routinely by commercial cattle producers, makes it difficult to identify subfertile bulls. For example, it is estimated that 20% to 40% of unselected beef bulls in North America are subfertile.[1] Because of this, there is increasing demand for breeding soundness evaluation, particularly for bulls destined for natural service. Knowledge of the anatomy, physiology, and behavior of bulls is necessary for veterinarians to conduct an adequate breeding soundness evaluation, investigate reproductive problems, and advise on reproductive management.

PRENATAL DEVELOPMENT

Sex determination in mammals occurs in three stages: the establishment of chromosomal sex, the development of primary sex characters (the gonads), and the subsequent development of secondary sex characteristics under the influence of gonadal hormones.

During embryogenesis, the gonad first arises from the mesonephros as undifferentiated tissue, which has the capability to subsequently develop in either male or female form. The precursors to the male reproductive tract system (i.e., the wolffian duct system) and the female tract (the müllerian duct system) are both present. Subsequent sexual differentiation is decided, in mammals, by the presence or absence of the Y chromosome, with females being XX and males XY. The presence of a Y chromosome results in male development, regardless of the number of X chromosomes present. Thus, the Y chromosome must contain the dominant inducer of testis formation; the testis determining gene (TDF or SRY). SRY is activated early in embryogenesis to commit the undifferentiated genital ridge to the testicular pathway. The early testis produces both testosterone (T; from the Leydig cells) and müllerian inhibiting substance (MIS; from the Sertoli cells). The latter induces the müllerian ducts to regress. Subsequent hormonal production induces male sexual differentiation. In the bovine, the differentiating gonad may be identified as a testis by 41 days after conception, with testosterone production (from fetal Leydig cells) evident soon thereafter.[2] By 3 to 4 months following conception, the testes have generally passed through the inguinal canal and entered the scrotum, which is derived from the urogenital folds. Although the basic components of a functional male gonad are present at birth, the spermatogonia do not undergo meiosis until the onset of spermatogenesis at puberty. Thus, the basic structure of the testis (seminiferous cords and interstitial tissues) remains much the same from early fetal life until the onset of puberty.

REPRODUCTIVE ANATOMY

The reproductive organs of the mature bull include paired testes, each with a spermatic cord, an epididymis, and a deferent duct (ductus deferens), which culminates in an ampulla.[3] In addition are paired vesicular glands, a prostate gland, paired bulbourethral (Cowper's) glands, and a fibroelastic penis, which incorporates a sigmoid flexure (Fig. 30-1).[4] The vesicular, bulbourethral, and prostate glands are often referred to as the accessory sex glands.

The testes are suspended within the scrotum, a feature that is important for testicular thermoregulation, as discussed subsequently. Within the testis, most (70% to 90% by weight) of the parenchyma is composed of seminiferous tubules (Sertoli cells and layers of germ cells). The remainder consists of interstitial tissue (Leydig cells, blood and lymph vessels, and connective tissue). The mediastinum, an area of connective tissue extending lengthwise in mid-testis, contains blood vessels and tubules of the rete testis. The testicular parenchyma is encased in a thick, connective tissue capsule (tunica albuginea), which is, in turn, covered by a thin, serous membrane (tunica vaginalis propria).[3]

In general, the spermatogenic efficiency of healthy testicular parenchyma in bulls is remarkably consistent (approximately $10–12 \times 10^6$ spermatozoa per gram daily). As testes weight is highly correlated with scrotal circumference ($r = 0.91$ to 0.98 in young beef bulls[1]), this highly repeatable measure has gained wide acceptance as an estimate of sperm-producing capability, especially as testicular size is heritable in beef bulls ($h^2 \sim 0.5$).[1] Although breed and environment may also influence testicular development, most variation in daily sperm production can be

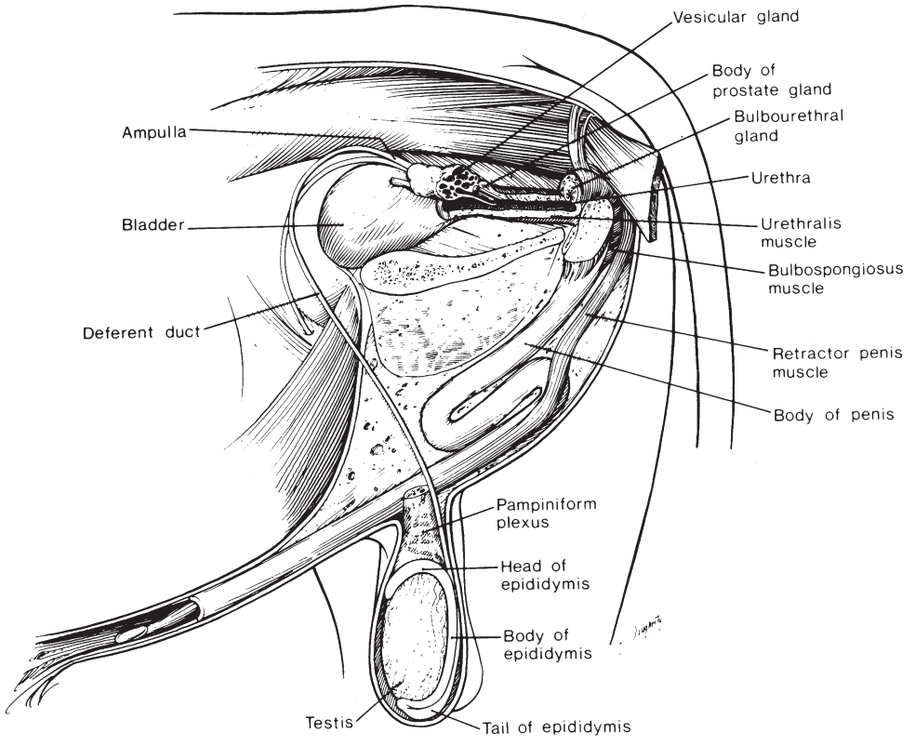

Fig. 30-1 Reproductive organs of the bull. (From Amann RP: How a bull works. Proceedings of the 11th Technical Conference on A. I. and Reproduction (NAAB), 1986, p 7.)

attributed to testicular size (represented by scrotal circumference), at least in younger (<4–5 years old) bulls.

Sertoli cells are amorphous, nucleated somatic cells that span the seminiferous epithelium and play a critical role in supporting and controlling germ cell development.[4] Specialized junctions between adjacent Sertoli cells form the blood-testis barrier, dividing the seminiferous epithelium into basal and adluminal compartments.[5] Spermatogonia lie between Sertoli cells and the tubule basement membrane, and other germ cells are located either in cytoplasmic crypts within Sertoli cells or sandwiched between adjacent Sertoli cells.

Spermatozoa are produced within the seminiferous tubules. Both ends of each tubule form tubuli recti (straight tubules), which join the rete testis (a complex of anastomosing spaces within the mediastinum testis). Spermatozoa pass from the rete testis to the head of the epididymis via the efferent ducts (n = 6–20). The epididymis, an elongated, torturous duct extending from the rete testis along the medioposterior border of the testis, comprises the head (caput), body (corpus), and tail (cauda) regions. Epididymal functions include sperm transport and maturation, as will be discussed.

At birth, the bull penis is short and slender and lacks a sigmoid flexure, and its apex is fused to the inner lining of the prepuce. With time (and under the influence of androgens), penile and preputial tissues separate, the penis elongates, and a sigmoid flexure develops. Tissue separation proceeds irregularly and in many bulls is completed only after the onset of erectile activity. Thereafter, incomplete separation is defined as a persistent penile frenulum (otherwise known as a persistent raphe or tied penis). This condition, most commonly present in Angus, Beef Shorthorn, Hereford, Polled Hereford, and Beefmaster bulls, probably has a genetic basis in many cases.[6] Although this condition is usually correctable with minor surgery, the consequences of perpetuating a genetic defect should be considered when this is done.

The prepuce is a double invagination of skin, with its internal lining everting upon penile erection to constitute much of the penile surface.[3] A fan-shaped protractor prepuce muscle raises and lowers the distal portion of the prepuce and also controls the size of the preputial opening. Retraction of the membrane lining the inner prepuce is under the control of the retractor prepuce muscle. Lack of development of this muscle (a condition genetically linked with the polled gene in bulls), predisposes to chronic eversion of this membrane with increased risk of traumatic injury.

The development and normal function of the accessory glands depend upon the effects of androgens. Castration results in marked depression of both development and secretory functions of these glands.

EPIDIDYMAL FUNCTIONS

The epididymis is far more than a passive organ, with functions including both sperm transport and maturation. Spermatozoa leaving the testis lack both the ability to survive in the female tract and to achieve unassisted fertilization. These capabilities are acquired in the epididymis. Other epididymal functions include the (1)

energy-efficient storage of sperm while maintaining sperm fertility; (2) intermixing of recently formed and older spermatozoa to provide a temporal spectrum of optimal sperm function, and (3) changing sperm attributes and environment to permit survival and ensure fertilizing capability within the female tract.[7]

Immotile spermatozoa are carried into the lumen of the seminiferous tubule following separation of their connections to Sertoli cells. Most of the residual cytoplasm is retained by the Sertoli cell, although some remains attached to the spermatozoa (as cytoplasmic droplets). Initial transport into the rete testis and caput epididymidis seems to be dependent upon fluid secreted by Sertoli cells. Once into the efferent ducts, sperm movement is facilitated by ciliated epithelial cells (within the ducts) as well as by smooth muscle contractions. The efferent ducts and initial segment of the caput epididymidis resorb most fluid and protein emanating from the testis and secrete new compounds.

Sperm maturation occurs within the caput and corpus of the epididymis with this process requiring the coordinated secretion of specialized enzymes and proteins. During this process, changes occur in the sperm DNA-protein complex, plasma membrane, mitochondria, axonemal complex, plasma and acrosomal membranes, and sperm surface characteristics.[7]

As spermatozoa progress through the epididymis, they achieve progressive motility, the cytoplasmic droplet migrates from the proximal to the distal position on the sperm midpiece, and seminal fluids are resorbed and exchanged. Heat stress can adversely affect epididymal function. The epididymis, particularly the tail region, acts as a storage region for sperm, such that a mature Holstein bull may have epididymal reserves representing the equivalent of 6 or 7 days of daily sperm output. Most sperm that are not ejaculated are voided in urine, with a small proportion resorbed by the male tract. Some evidence exists for selective resorption of spermatozoa within the epididymis.

Sperm are transported from the cauda epididymis to the urethra in the ductus deferens (vas deferens) via muscle contractions that are strongest during precoital stimulation. The terminal portions of the ductus deferens expand to form the ampullae (each approximately 10 × 1.5 cm in adult bulls). These ampullae act as minor sperm storage areas and they also secrete fructose and citric acid into the seminal plasma. The ductus deferens open (via the ampullae) into the cranial portion of the pelvic urethra. The vesicular glands (or seminal vesicles) also open into the pelvic urethra. These glands provide much of the fluid component of the bull ejaculate, as well as sperm nutrients and semen buffers. The vesicular glands are lobulated organs, approximately 10 to 15 cm in length and 2 to 4 cm in diameter in mature bulls. The prostate gland, consisting of a relatively small body and larger disseminate region, produces 25% to 40% of seminal volume as well as semen odor. The bulbourethral glands of the bull each open into the pelvic urethra at the ischial arch. The urethra is an elongated tube extending from the bladder to the tip of the penis. It is surrounded by the urethralis muscle, which contracts strongly during ejaculation.

SCROTAL TESTICULAR THERMOREGULATION

Scrotal testicular thermoregulation has been recently reviewed.[8] Testicular temperature in bulls must be 2 to 6°C cooler than core body temperature for effective production of fertile spermatozoa. Several mechanisms act to regulate the testicular temperature. Of major importance is the pampiniform plexus, a complex venous network that surrounds the highly coiled testicular artery within the neck of the scrotum. This entire structure (vein and artery) is properly called the testicular vascular cone.[8] The cone functions as a countercurrent heat exchanger whereby heat is transferred from arterial to venous blood. Scrotal skin is thin and relatively hairless, with extensive blood vessels that can dilate to increase heat loss. The testes and scrotum have complementary temperature gradients that result in a nearly uniform intratesticular temperature.[8] Other mechanisms that help to cool the testes include relaxation of scrotal muscles, scrotal sweating, and whole-body responses (e.g., panting and peripheral vasodilation). Spermatogenesis occurs in an environment that verges on hypoxia. When testicular temperature increases, spermatogenic metabolism increases faster than does blood flow, and hence the testes become hypoxic.[8] Therefore, testicular function is very susceptible to temperature increases due to either endogenous or exogenous factors (e.g., fever or high ambient temperatures, respectively). Increases in testicular temperature cause increased production of defective spermatozoa. The proportion of defective spermatozoa and the time required for recovery depend upon the nature and duration of the thermal insult.[8] Severe insult may cause irreversible spermatogenic damage.

ENDOCRINOLOGY

The function of male reproductive organs is under the control of both the nervous and endocrine systems. The latter includes the hypothalamus, anterior pituitary, and the Leydig and Sertoli cells. The hypothalamus plays a pivotal role in the control of reproduction, acting as the interface between the nervous and endocrine systems. It works within a complicated feedback system (the hypothalamic-pituitary-gonadal complex or axis), which is ultimately controlled by the inputs from the higher nervous system. In turn, this can be influenced by many factors, both environmental and behavioral.

Gonadotropin-releasing hormone (GnRH) from the hypothalamus travels (via the portal system) to the anterior pituitary gland where it stimulates the synthesis and release of both LH (luteinizing hormone) and FSH (follicle-stimulating hormone). Leydig cells, located within the interstitial tissue (in close apposition to lymph and blood vessels), produce episodic bursts of T (testosterone) in response to LH release. T is concentrated in the seminiferous tubules, testicular lymph, and venous blood via a vascular-concentrating mechanism situated within the scrotal cord.[2] T is necessary for Sertoli cell function, and both androgens and FSH maintain spermatogenic function.[2] In addition, T is necessary for the development and function of accessory glands as well as epididymal

function, secondary sex characteristics, and mature sexual behavior. Although libido is apparently not quantitatively associated with resting T concentrations, it appears that a threshold level is necessary for activation. In response to FSH, Sertoli cells secrete fluids and androgen-binding-protein (ABP) (thereby indirectly influencing spermatogenesis).[2] Sertoli cells also produce inhibin and activin, which inhibit and stimulate FSH secretion, respectively, as well as proteins that preferentially target spermatocytes and early spermatids. Leydig cells also produce estradiol (E), which acts in a similar manner to T by causing negative feedback on both the hypothalamus and anterior pituitary, thus suppressing the release of GnRH, LH, and FSH (Fig. 30-2).

Recently, the role of growth factors has been investigated in relation to male reproductive processes. Such factors act by stimulating target cell proliferation and regulate growth of reproductive organs. They include cytokines, interferons, insulin, insulin-like growth factors (IGFs), and others such as platelet-activating-factor (PAF) and epidermal growth factor (EGF). In the male, such factors have been associated with epithelial and interstitial cell development and function, puberty, LH modulation, and sperm motility. This represents a dynamic research area, with new findings appearing regularly to improve our understanding of the fine tuning of male reproduction.

PUBERTY

Puberty in bulls is a process that implies the attainment of functional sexual organs and behavior. Sexual maturity occurs when the development of both spermatogenesis and reproductive behavior allow effective coordinated service and subsequent fertilization. However, such definitions obscure the fact that puberty is a continuous and dynamic process, which commences prior to birth and is mediated through the hypothalamic-pituitary axis.

At birth, the testes are small, solid, and composed of cords of gonocytes and undifferentiated supporting cells. In prepubertal bulls, there is an early rise in gonadotropins (in particular LH and less consistently FSH) between 10 and 20 weeks of age.[9] The earlier this rise in gonadotropins occurs, the earlier the onset of puberty.[9] Spermatogonia start to appear in tubules within approximately 8 to 14 weeks after birth, with spermatocytes appearing shortly afterward. Seminiferous tubules form lumens between 15 and 40 weeks. Sequential maturation of spermatogonia through primary and

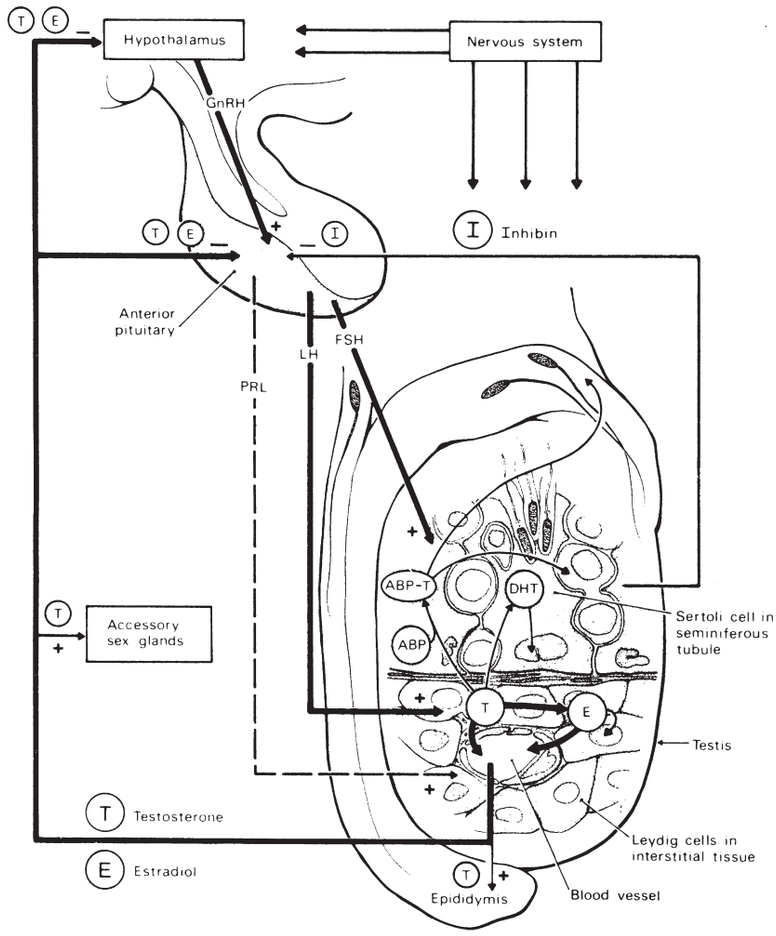

Fig. 30-2 Endocrinology of bull reproduction, showing the interrelationship of hormone production in the Leydig cells and seminiferous tubules and the feedback control of gonadal hormones on the hypothalamus and anterior pituitary glands. ABP, androgen binding protein; E, estradiol or other estrogens; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; I, inhibin; LH, luteinizing hormone; PRL, prolactin; T, testosterone. (From Amann RP: How a bull works. Proceedings of the 11th Technical Conference on A. I. and Reproduction (NAAB), 1986, p 17.)

secondary spermatocytes to spermatids and spermatozoa is achieved between weeks 32 to 44 in well-fed *Bos taurus* breeds.[10] Testicular growth is very rapid between 7 and 10 months of age. Blood concentrations of androgens (initially androstenedione and then testosterone) start to increase at about 6 months of age and continue to rise through puberty (until at least 13 months of age).[10] Puberty is often defined as the first time a bull produces an ejaculate with at least 50×10^6 spermatozoa per milliliter with at least 10% progressive motility.[1] Increases in sperm concentration and the proportion of morphologically normal spermatozoa occur in conjunction with decreasing proximal droplets for at least 4 months after puberty.[5] Although breeds vary in both testicular development and body weight, puberty is quite consistently achieved when scrotal circumference reaches approximately 25 to 27 cm.[1] Therefore, scrotal circumference is generally a better predictor of puberty than either age or body weight, regardless of breed.[10] However, breed differences do occur in the pattern of testicular development. Bull age strongly influences scrotal circumference in young well-grown *Bos taurus* breeds, whereas body weight tends to be more predictive in *Bos indicus* breeds, especially when nutrition is suboptimal. Postweaning average daily gain does not appear to be highly related to scrotal circumference in bulls.[1] However, high-energy diets may depress both libido and spermatogenesis in young bulls, and an association has been reported between excessive backfat and lowered fertility.[1] Prepuberal scrotal circumference measures in bulls have not proved to be reliable predictors of subsequent postpuberal testicular size, although use of minimum thresholds show promise. For example, one study estimated that, to obtain a 30-cm scrotal circumference by 365 days of age, Angus, Simmental and Zebu-derived bulls needed to have a scrotal circumference of 23 cm at start of a 140-day growth test, while other continental breeds and Polled Herefords required 26 cm.[1] Favorable associations with scrotal circumference in beef bulls include age at puberty in related heifers[1] and improved semen quality.[1]

SPERMATOGENESIS

Spermatogenesis is the sum of cellular transformations that occur within the seminiferous epithelium and result in the production of spermatozoa (Fig. 30-3). The process of cellular differentiation commences with spermatogonial stem cells and proceeds through several mitotic divisions, a meiotic division, and numerous cytologic transformations, before culminating as elongated

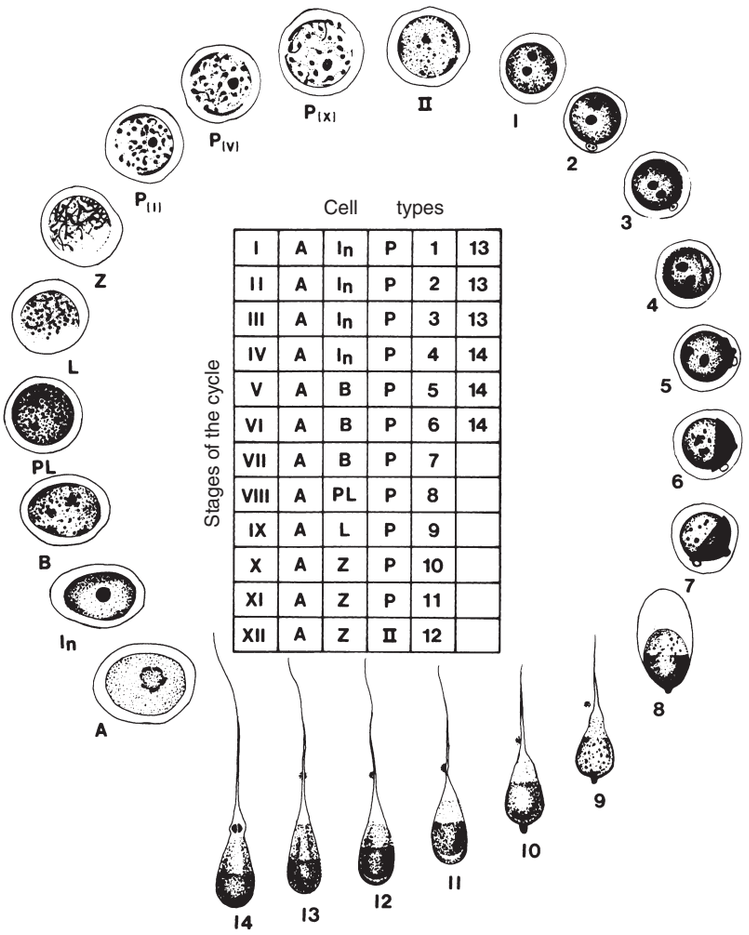

Fig. 30-3 Spermatogenesis in the bull. Diagrammatic representation of a 12-stage categorization of the cycle (I–XII) and the cell types involved including spermatogonia (A, In, and B), primary and secondary (II) spermatocytes, and the different stages of spermiogenesis (1-14), showing maturational acrosomal and morphologic changes. L, leptotene; P, pachytene; PL, preleptotene; Z, zygotene. (Adapted from Berndtson WE, Desjardins C: 1974. From Hafez ESE (ed): *Reproduction in farm animals,* 5th ed. Baltimore: Williams & Wilkins, 1987.)

spermatids.[2–5] Knowledge of the structures, processes, and temporal relationships of spermatogenesis is essential for informed diagnosis and prognosis of problems. Some relevant terms and definitions are as follows:

- **Seminiferous (germinal) epithelium**—the cellular constituents within the seminiferous tubule, comprising Sertoli cells, spermatogonia, primary and secondary spermatocytes, and spermatids. The spermatogenic cells become more highly differentiated as they move from the basement membrane toward the tubular lumen.
- **Stages of the seminiferous epithelium**—a series of discrete cellular associations within the seminiferous epithelium (8 to 12 stages are commonly described in domestic animals) that recur in a consistent sequence and under strict temporal regulation. In bulls, 12 stages are often described, with the duration of each stage ranging from approximately 2 hours to 2 days.[2,5]
- **Cycle of the seminiferous epithelium**—the complete sequence of stages appearing at any given site within the seminiferous epithelium.
- **Duration of the cycle**—interval required for one complete cycle of the seminiferous epithelium (time required for one stage to recur at a given site). The duration of the cycle in bulls is approximately 13.5 days.[2]
- **Duration of spermatogenesis**—interval required for a stem spermatogonium to transform into an elongated spermatid, ready for release (spermiation) into the tubular lumen (approximately 61 days in bulls).[5]
- **Epididymal transit time**—the interval between release and ejaculation. In bulls, the duration of this interval averages 8.3 days (range, 7 to 13 days), with shorter periods occurring in response to frequent ejaculation.[2]
- **Daily sperm output (DSO)**—number of sperm harvested per day from a bull. DSO is not only a function of sperm production, but it is also influenced by ejaculation frequency, bull stimulation, and collection method.
- **Daily sperm production (DSP)**—the total number of sperm produced per day by the testes.[2] DSP is usually assessed by histologic examination of testicular tissue or by preparing a homogenate and counting cells. A less invasive approach is to determine total sperm in ejaculates obtained from males in which extragonadal sperm reserves (EGR) have been stabilized by frequent ejaculation. Under these conditions, DSO can approximate DSP, which is typically 7 to 9×10^9 spermatozoa for mature Holstein bulls and somewhat less for beef and *Bos indicus* breeds.

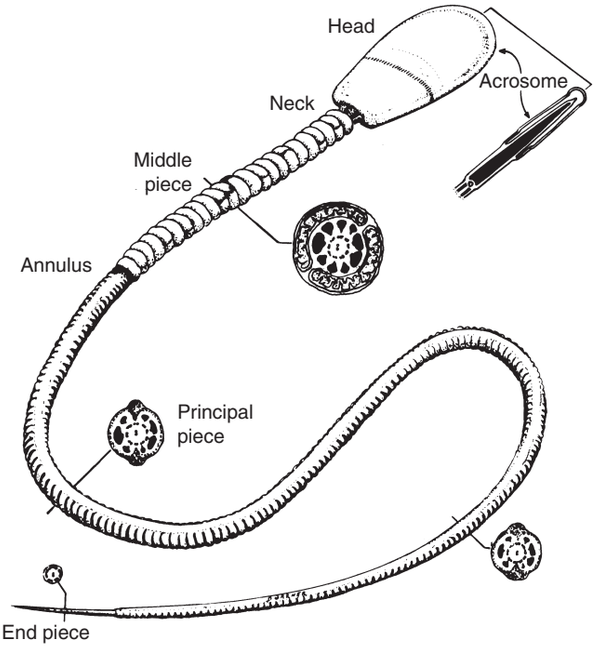

Fig. 30-4 The major regions (head, midpiece, and tail) of the bovine spermatozoon. Sagittal sections show the relationship between the acrosome and sperm head as well as structural details of the principal piece and its relationship to the mitochondrial sheath of the midpiece. (From Hafez ESE (ed): *Reproduction in farm animals,* 5th ed. Baltimore: Williams & Wilkins, 1987.)

SEMEN AND SPERMATOZOA

Bovine semen consists of spermatozoa suspended in seminal plasma. The latter is derived from multiple sources, including the testes, epididymides, and accessory glands (with a substantial proportion being provided by the vesicular and prostate glands). Seminal plasma contains sperm metabolites (including fructose, citric acid, sorbitol, glycerylphosphorylcholine and inositol), amino acids, enzymes, antimicrobials, hormones, and immunoglobulins.[11]

Bull spermatozoa are similar in structure to those of other domesticated mammals (Fig. 30-4). They consist of a flattened head, midpiece, and tail (with principal and end pieces).[5,11] The sperm head is a specialized package of condensed chromatin containing DNA, surrounded by a nuclear membrane, with an acrosome on its anterior aspect. The presence of an intact acrosome is an essential prerequisite for fertilization as it is the site of capacitation and the acrosome reaction. The equatorial region and the anterior border of the postacrosomal region (postnuclear cap) represent the sites of sperm fusion with the oocyte.[11] Sperm motility derives from movement of microtubule doublets within the axonemal complex, with energy supplied mainly by oxidative mechanisms within mitochondria, which form a helical mitochondrial sheath (known as the sperm midpiece) that surrounds the proximal portion of the axonemal complex.

SPERM TRANSPORT AND EJACULATION

Immotile spermatozoa are carried into the lumen of the seminiferous tubule following separation of their connections to Sertoli cells within the spermatogenic epithelium. Most of the residual cytoplasm is retained by the

Sertoli cell, although some remains attached to the spermatozoa (as cytoplasmic droplets). Initial transport into the rete testis seems to be dependent upon fluid secreted by Sertoli cells. Once into the efferent ducts, sperm movement is facilitated by ciliated epithelial cells (within the ducts) as well as by smooth muscle contractions. The efferent ducts and initial segment of the caput epididymidis resorb most fluid and protein emanating from the testis and secrete new compounds.[2]

Transportation of spermatozoa within the epididymis is primarily due to smooth muscle contractions. As spermatozoa progress through the epididymis, they achieve the ability to be progressively motile, the cytoplasmic droplet migrates from the proximal to the distal position on the sperm midpiece, and fluids are resorbed and exchanged. Through these and other changes, the epididymis plays a pivotal role in preparing sperm for fertilization.[2] The availability of testosterone, particularly in the initial and middle segments of the epididymis, is an important factor in sperm maturation. Heat stress can adversely affect epididymal function. The epididymis, particularly the tail region, acts as a storage region for sperm, such that a mature Holstein bull may have epididymal reserves representing the equivalent of 6 or 7 days of daily sperm output. Most sperm that are not ejaculated are voided in urine, with a small proportion resorbed by the male tract. There is some evidence for selective resorption of spermatozoa within the epididymis.

Sperm are transported from the cauda epididymidis to the urethra in the ductus deferens (vas deferens) via muscle contractions that are strongest during precoital stimulation. The terminal portions of the ductus deferens expand to form the ampullae (each approximately 10 × 1.5 cm in adult bulls); these act as minor sperm storage areas as well as secreting fructose and citric acid into the seminal plasma. The ductus deferens open (via the ampullae) into the cranial portion of the pelvic urethra. The vesicular glands (seminal vesicles) also open into the pelvic urethra, providing much of the fluid component of the bull ejaculate, as well as sperm nutrients and semen buffers. These lobulated glands are approximately 10 to 15 cm in length and 2 to 4 cm in diameter in mature bulls.[3] The prostate gland, consisting of a relatively small body and larger disseminate region, produces 25% to 40% of seminal volume as well as semen odor. The bulbourethral glands of the bull each open into the pelvic urethra at the ischial arch. The urethra is an elongated tube extending from the bladder to the tip of the penis. It is surrounded by the urethralis muscle, which contracts strongly during ejaculation.

The penis of the bull is fibroelastic with erection causing relatively little increase in penile diameter.[3] Retraction of the penis into the sheath is controlled by the retractor penis muscle. Ejaculation coincides with the penis reaching its maximal length and momentary peak pressure within the corpus cavernosum penis (CCP) as high as 14,000 mm Hg; the pressure is due to vascular engorgement of the CCP caused by rhythmic contractions of the ischiocavernosum muscle, forcing blood anterior from both crura. Ejaculation lasts approximately 1.3 seconds and is often accompanied by coiling of the penis. The ejaculatory thrust of the bull is achieved primarily through abdominal muscle action and is triggered by vulvar stimulation of nerve endings located just posterior to the glans penis.[12]

SEXUAL BEHAVIOR

Reproductive behavior in the bull has been recently reviewed.[13] In the breeding herd, the female plays a major role in the solicitation of sexual partners and also in controlling sexual activity. Females in proestrus or estrus congregate to form a sexually active group, which usually stays within visual contact of the bull (or bull group). The bull is attracted by the sight of mounting activity, particularly if the mounted female displays immobility.[12] Here, visual cues are usually of greatest importance to the bull, with olfactory cues playing a secondary role. The bull may test the female for immobility by chin resting and sham mounting attempts.[13] When more than one female is receptive, the bull will often preferentially mount the one that most recently came into estrus. Bulls may service an estrous female repeatedly, depending upon his libido (sex drive), stimulus pressure, and the length of time that the female remains receptive.[13]

Bulls can vary considerably in libido and breeding capability (ability to successfully mount and breed), with both traits having strong genetic influences. Modern management systems, whether employing natural or artificial breeding, may perpetuate deficiencies in bull libido and mating ability. Breeding capability also has a learning component, some of which is apparently acquired via mounting activity by juveniles. Despite such juvenile behavior, young bulls raised in bachelor groups often show signs of sexual inexperience when first placed with females. In multi-sire breeding pastures, the sexual activity of bulls can be strongly influenced by their social position in the bull group. Here, more dominant males may monopolize estrous females even though they might have poor libido, mating ability, or semen quality. This situation can result in reduced herd fertility.[13]

Bull libido is subject to various influences including physical problems such as lameness, obesity, presence of a hernia, penile abnormalities, and illness.[12] High-energy diets may reduce bull libido, whereas underfeeding is probably deleterious only when severe enough to affect the physiologic well-being of the bull.[12]

Serving capacity is a measure of the number of services achieved by a bull under defined conditions and thus includes aspects of both libido and mating ability. Testing methods have been developed to quantify both libido and serving capacity.[12,13] Although secretion of steroid hormones initiates sexual motivation, good relationships between circulatory levels of these hormones and quantitative estimates of sexual behavior in bulls have proved to be elusive.[13] Bull age and sexual experience can influence relative mating efficiency and hence libido scores and rankings.[13] A learning component for breeding capability of young bulls has been described in some studies, although inexperience is usually rapidly overcome following adequate exposure to females. More research is needed to differentiate the effects of age and inexperience on bull libido and breeding capability from environmen-

tal and managerial influences. Test scores for libido or serving capacity have generally shown poor relationships with breeding soundness scores, scrotal circumference, semen quality, size of vesicular glands, dominance rank, average daily gain, and resting blood concentrations of either testosterone or LH.[13] This indicates that, with the current state of knowledge and technology, comprehensive bull evaluation will require assessment of physical soundness, internal and external genitalia, semen quality and libido, as well as his breeding capability.

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CHAPTER 31

Evaluation of Potential Breeding Soundness of the Bull

ALBERT D. BARTH

It is estimated that at least one in five bulls in an unselected population would be subfertile owing to inability to serve cows efficiently or to poor semen quality. Although some bulls have such serious impediments to fertility that low pregnancy rates result, many bulls that are less than satisfactory achieve acceptable pregnancy rates under low breeding pressure and over a prolonged breeding season. Disastrously low pregnancy rates are very noticeable and can be financially devastating to the individual producer. In the beef industry, the greatest economic loss that is attributable to subfertile bulls is delayed conception (low first-service pregnancy rate)—which may not be noticed by the producer. It has been estimated that for every 21 days of the breeding season during which a cow remains open, a loss of 50 to 60 pounds of weaning weight can be expected the following year for the calf she finally conceives. Therefore, a subfertile bull could be the cause of economic losses in the range of $1500 to $3000 due to reduced weaning weights a year later, depending on the degree of subfertility, bull-to-female ratios, and weaned calf prices. Additional losses will accrue with the culling of open cows and cows that conceived late. Cows that calve late tend to do so perpetually in following years.

In multiple-sire pasture breeding, a great deal of breeding overlap will occur. As many as 80% of cows may be bred by two or more bulls during one estrous period. Thus, bulls with high fertility can compensate for bulls with poor fertility. Because of the common use of multiple-sire breeding and low bull-to-female ratios, inept bull evaluation often is masked by the excellent performance of highly fertile bulls in a group. Nevertheless, as the

tal and managerial influences. Test scores for libido or serving capacity have generally shown poor relationships with breeding soundness scores, scrotal circumference, semen quality, size of vesicular glands, dominance rank, average daily gain, and resting blood concentrations of either testosterone or LH.[13] This indicates that, with the current state of knowledge and technology, comprehensive bull evaluation will require assessment of physical soundness, internal and external genitalia, semen quality and libido, as well as his breeding capability.

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In multiple-sire pasture breeding, a great deal of breeding overlap will occur. As many as 80% of cows may be bred by two or more bulls during one estrous period. Thus, bulls with high fertility can compensate for bulls with poor fertility. Because of the common use of multiple-sire breeding and low bull-to-female ratios, inept bull evaluation often is masked by the excellent performance of highly fertile bulls in a group. Nevertheless, as the

economic pressure for highly efficient beef production increases, including the use of short breeding seasons, high bull-to-female ratios, and single-sire breeding groups, the need for thorough, unbiased breeding soundness evaluations also will increase.

Veterinarians often feel pressured to compromise their standards by clients who wish to obtain a "satisfactory" classification for breeding soundness of certain bulls (discussed later in the chapter under "Breeding Soundness Classification"). A veterinarian's fear of jeopardizing client relationships and lack of self-confidence regarding ability to evaluate bulls may result in approval for use of bulls of questionable fertility. In the long term, taking chances on cow herd fertility is of benefit to no one. Although it may be difficult to be sure that cow fertility will actually be depressed by allowing mating with questionable bulls, only bulls with the potential for high fertility should be classified as satisfactory.

In addition to freedom from disease, a bull requires three attributes to be fertile: (1) good libido, (2) physical soundness, and (3) good semen quality. These three attributes must be held in the forefront of all decisions regarding herd sire selection and breeding soundness evaluations.

LIBIDO AND SERVING CAPACITY

Serving capacity describes the number of matings a bull is willing and able to perform in a test situation. In spite of extensive research showing the importance of libido and serving capacity to fertility, the use of standardized tests has not become part of routine bull evaluation in North America. Furthermore, methods of bull evaluation have in the past almost totally ignored this part of breeding soundness, and producers often have been lulled into a false sense of security by a satisfactory test result. Veterinarians must inform producers when this critical component of bull fertility remains unassessed. It is particularly important to assess libido and serving capacity in yearling and 2-year-old bulls that have had no previous breeding experience. Many factors, however, including injuries and the development of penile deviations, can incapacitate older bulls that have been sound breeders in the past.

It may be necessary to advise producers on how to assess bull libido and serving capacity. For example, the mere presence of an older dominant bull may completely suppress the expression of libido in younger bulls. Yearling bulls often require some time after exposure to estrual females to develop their instincts and learn how to become efficient breeders. Studies have shown that libido or serving capacity tests do not provide reliable results in yearling bulls. Therefore, observations for libido and serving capacity must be diligently undertaken at the beginning of the breeding season and continued for its duration to ensure that estrual females are being bred.

Under certain circumstances, whether to assess the libido and serving capacity of individual problem bulls or for selection of a bull battery for a producer's herd, practitioners may wish to become involved in libido and service capacity testing. Various testing methods have been investigated, including pasture versus corral settings, use of restrained versus unrestrained cows, use of estrual versus nonestrual cows, test durations ranging from 5 minutes to days, and testing each bull individually versus four or five bulls simultaneously. In Australia, a 3-hour corral test with restrained heifers had a correlation of 0.92 with a 19-day pasture test, but because the first hour of that test had a correlation of 0.91, 1 hour was considered satisfactory. Further testing demonstrated that a 40-minute test had a correlation coefficient of 0.99 as compared with 1 hour. Reduction to a 20-minute test period lowered the correlation to 0.91. Twenty-minute tests seem to be the most practical and are of sufficient accuracy for most purposes.[1]

Methods of Testing Libido and Serving Capacity

Bulls to be tested are exposed to restrained cows in a small paddock or pen and observed for expression of sex drive, ability to serve cows, and the number of services completed in a given time period. Strong cows in good body condition are preferred as mount animals. One or more mount cows are restrained in short-sided breeding crates and sedated with xylazine (0.03 mg/kg IV). Cows need not be in estrus, and some practitioners believe that bulls spread out their services more evenly if cows are nonestrual and restrained at least 20 meters apart. When only one or two bulls need to be tested, it usually is advantageous to have an estrous cow to ensure sexual stimulation of the bulls to be tested. The vagina of each mount cow should be lubricated with approximately 30 ml of a sterile lubricating jelly to reduce tissue trauma from repeated breedings.

Bulls to be tested must first receive 10 minutes of sexual stimulation by allowing them to watch another "stimulator" bull serving the mount cow. The stimulator bull may be a test bull himself, and if he performs well initially, without prior sexual stimulation, further testing may not be necessary for him. Generally it is best to select an older, experienced bull to be the stimulator bull. Depending on available human assistance, one or two more bulls than mount cows are turned into the pen with the mount females. For example, with two mount cows, three bulls may be turned into the test pen at one time. Personnel can encourage serving by moving individual bulls to a different cow, separating bulls that are interfering with each other, or removing bulls that appear to be a threat to more timid bulls. Younger bulls should be tested separately from older bulls to prevent social dominance factors from invalidating test results. Some bulls are afraid of people and will not perform in a test situation. Bulls that give questionable results should be retested under different conditions or on different days to ensure that good bulls are not culled erroneously owing to spurious test results. Provided that bulls do not develop health problems, serving capacity test results are highly repeatable. Even after a period of 6 months, bulls had remarkably similar serving capacity scores.

In a 20-minute test, a serving capacity of 0 to 1 is considered low, 2 to 3 is medium, and 4 or more services are considered to indicate high serving capacity. When large numbers of bulls need to be tested, and only bulls of low

serving capacity will be culled, bulls that serve a cow twice within the first few minutes can be removed immediately, to conserve the mount cows and speed up the testing process.

PHYSICAL SOUNDNESS

General Health

Breeding soundness examinations are not meant to be health examinations. It is taken for granted that bull health, like the health of all other herd members, is the concern of the producer, who is responsible for detecting any signs of health problems. By virtue of their training, however, clinicians should intuitively be alert for abnormalities in a bull's attitude, general appearance, body condition, and fecal characteristics that may warrant further investigation into the health of the animal presented for fertility evaluation. Because bulls rely mostly on vision to detect cows in heat, and because the development of squamous cell carcinoma or corneal opacity due to pinkeye is common, special emphasis is placed on examining the eyes. Furthermore, some evidence suggests that squamous cell carcinoma has a heritable basis, and selection against it may be well worthwhile to reduce the incidence of this problem in a cow herd.

The Musculoskeletal System

A great deal of emphasis must be placed on sound conformation of the feet and legs of a bull. Bulls must travel long distances in order to detect females in heat and breed them. During the mating act, the full weight of the bull (commonly 900 to 1100 kg [1980–2420 lbs]) is borne on the hind legs and feet. Clearly, then, any unsoundness in this region will drastically interfere with breeding ability. Many producers fail to realize that although a bull with a conformation defect of the feet or legs may get by for 2 or 3 years, the more serious danger is that the defect will be passed on to offspring. Replacement heifers carrying conformational defects will propagate these problems in the cow herd, reducing longevity, increasing labor in herd management, and increasing veterinary expense. Common foot and leg problems that have a hereditary basis include the corkscrew claw defect, interdigital fibromas (corns), weak pasterns, post-leggedness, and sickle hocks. An effort must be made during the breeding soundness evaluation to watch the bull walk. Too often bulls are seen only standing in a chute, and although most of the onus is on the breeder to cull bulls with poor conformation and on the producer to procure physically sound bulls, it is not uncommon for them to disregard or fail to detect serious abnormalities.

The Scrotum and Testes

Examination of the Scrotum and Its Contents

Bulls must be adequately restrained to allow comfortable and thorough palpation of the scrotum and its contents. If a clinician does not feel safe behind the bull, a careful examination cannot be completed. For most *Bos taurus* bulls, a strong pole placed at the proper height tightly against the bull's lower buttocks will allow examination of the scrotum and yet prevent injury from kicking. A height of 30 inches (76 cm) from the floor to the bottom of the pole is most practical and works well for most sizes of bulls. *Bos indicus* bulls generally require more secure restraint systems.

Letting the bull become aware of the veterinarian's presence behind him by talking to him and touching him on the rump and thighs before touching the scrotum usually will prevent the bull from reacting to scrotal examination. In general, bulls do not resent palpation of the testes and rarely kick; however, it is common for them to lift their legs at the beginning of the examination. With such movements, the bull's hock may catch the veterinarian's arm and knock it against the post above. To prevent this, one arm should be introduced between the bull's hind legs parallel to the bull's longitudinal axis at the start of palpation until the bull settles down. When both arms are placed between the bull's hind legs, the elbows should be held together. Bulls also are likely to flinch or lift their feet during forceful pressing of the testicles to the bottom of the scrotum. If large numbers of bulls must be examined, padding the veterinarian's wrists and forearms may be helpful. Some bulls are very fractious, and palpating the scrotum may be dangerous because of continuous balking at restraint in the chute. If semen collection is planned, it often helps to do the semen collection first. After the procedure of electroejaculation, fractious bulls usually stand quietly for the scrotal examination.

A visual appraisal of the shape of the scrotum in a warm environment, while the bull is relaxed, reveals valuable information about the thermoregulatory abilities of the scrotum, as well as giving an indication of testis size. Presence of a scrotal "neck" above the testes is of critical importance because this region contains the countercurrent heat exchange mechanism of the testicular cords. In cooler temperatures, the scrotal shape cannot be determined, because the dartos muscle in the scrotal wall and the cremaster muscles will hold the testes closer to the body wall. In cool conditions, the testes must be manually pushed down into the scrotum, stretching the puckered scrotal wall to allow an assessment of scrotal shape. Nevertheless, it is often difficult to reliably assess scrotal shape in cool temperatures. Short scrotums or excess fat in the scrotal neck will prevent normal heat exchange, resulting in abnormal spermatogenesis and possibly testicular degeneration. Less commonly, abnormal scrotal shape may be due to a short caudal frenulum of the scrotum, unilateral testicular hypoplasia, orchitis, scrotal hernia, rotation of the testis, displacement of the cauda epididymidis, or rotation of the scrotum so that the testicles are held in tandem. The scrotum also should be examined for thickness of the scrotal wall, the amount of fat in the neck of the scrotum, and lesions in or on the scrotum.

The testicular cords should be palpated from the body wall down to the top of the testes to detect abscesses, variceles, or a scrotal hernia. The caput epididymidis, located primarily craniodorsally on the testis, usually is palpable and may feel more prominent in some bulls than in others. It is not uncommon to find enlargements

in this area due to inflammation or sperm granulomas, which may prevent sperm transport and result in a small, flaccid, empty cauda epididymidis. The body of the epididymis can be palpated on the medial aspect of the testis by first sliding the opposite testis upward; however, it is extremely rare to detect abnormalities in the corpus epididymidis. The cauda epididymidis of a normally functioning testis is turgid and prominent. Differences in size and consistency between the left and the right cauda epididymidis may indicate inflammation on one side or may result from a blockage of sperm transport on the side of the smaller cauda. Segmental aplasia of one or both epididymides probably is an inherited condition.[2] Occasionally, the ligament that attaches the cauda epididymis to the bottom of the testis is absent or very long, so that the cauda is separated from the bottom of the testis. This condition does not necessarily interfere with semen quality and may not be of concern to a commercial cow-calf producer.

The testes must move freely within the scrotum. Careful palpation of the testes must be done to detect possible abscesses, tumors, hematoceles, or calcification. In some cases, ultrasonography or infrared thermography may be helpful for diagnosis of testicular abnormalities. The consistency of the testis often is difficult to ascertain by subjective palpation. Although the use of tonometers for measuring consistency of testes removes much of the subjectivity, tonometer measurements have not been strongly correlated with semen quality and are not commonly used. In general, yearling bulls have very firm testes compared with those of older bulls. Testes that are obviously soft are most likely to indicate testicular degeneration. This usually can be confirmed by semen analysis.

Scrotal Circumference

The relationship of scrotal circumference to fertility. Scrotal circumference (SC) measurements are highly correlated with paired testis weight, which in turn is directly and highly correlated with daily sperm production and high semen quality traits.[3] Considerable evidence also indicates that SC measurements between 1 and 2 years of age are moderately to highly heritable.

Studies have shown that SC is a more accurate predictor of age at onset of puberty than either age or weight, regardless of breed. Among breed groups, negative correlations greater than 0.9 have been observed between SC and bull age at puberty, age at puberty in half-sibling heifers, and age at puberty in heifer offspring. Good evidence indicates that heterosis in cattle for traits related to size and age at puberty in females and SC of males is due to dominance effects of genes. Furthermore, correlations of 0.66 and 0.97 have been found between breed mean SC and fertility of female offspring.[4]

Strong genetic correlations were reported between SC and age at first breeding (–0.77), age at first calving (–0.66), and pregnancy rate (0.66). Because age at puberty in females is favorably associated with subsequent reproduction, selection for larger SC should improve the reproductive potential of the cow herd.

There is no question that small testes are undesirable. Histologic studies in 14-month-old bulls and in 2- to 3.5-year-old bulls showed that the proportion of seminiferous tubules with normal seminiferous epithelium is significantly lower in small testes (SC less than 32 cm). Yearling bulls with small testes do not exhibit catch-up growth over time and will have small testes at 2 years of age as well. Therefore, yearling bulls with SC below the recommended minimum should be culled.

Effects of nutrition. In young bulls, scrotal circumference measurements are affected by breed, body weight, and age at onset of puberty. Testis growth rate is maximal during puberty, and the level of nutrition in young growing bulls has a great influence on the age at onset of puberty. High-energy diets with adequate protein, vitamins, and minerals hasten the onset of puberty in bulls.[5] The early attainment of puberty improves the opportunity for early postpubertal development. This implies greater numbers of and higher-quality spermatozoa available when the bull is first used for breeding.

High-energy intakes up to about 12 months of age in beef bulls usually do not impair future semen quality, provided that their rations from 1 to 2 years of age do not result in fattening. This probably is because young, rapidly growing bulls can put the excess energy into growth, rather than fat. Nevertheless, Angus and Hereford bulls fed a high-energy diet of 80% grain and 20% forage from weaning to 15 months of age showed significantly lower sperm outputs than those in bulls on a medium-energy diet of 100% forage. Bulls in high-energy diet groups had a greater mean SC at 12 months, but not 15 months, of age, than that in bulls in medium-energy diet groups. Furthermore, excessive energy intake in young bulls may result in abnormal foot growth and conformation because of laminitis and possibly epiphysitis. In addition, high-energy diets increase the risk of rumenitis and liver abscesses, which may lead to the development of vesicular adenitis and epididymitis.[6]

Twelve-month-old Hereford and Angus bulls fed high-energy diets (80% concentrate) until 21 months of age had reduced epididymal sperm reserves, lower percentages of progressively motile sperm, and higher percentages of sperm abnormalities compared with bulls fed a medium-energy diet. Furthermore, Hereford bulls on the high-energy diet demonstrated a decline in testicular size that began at approximately 19 months of age, probably from testicular degeneration brought on by obesity.

Effect of breed. The spermatogenic rates in normal postpubertal bulls of different breeds are similar, and each gram of functional seminiferous tissue contains a similar amount of seminiferous tubule epithelium. Different studies have shown that each gram of normal functioning testicular tissue produces approximately 17 million sperm per day. Testicular shape is remarkably uniform among all breeds. To date, it has not been proved that some breeds with longer, narrower testes can produce larger numbers of sperm than other breeds with equivalent SC measurements.

Significant genetic variation exists among breeds of beef cattle for age at puberty.[7] In general, faster-gaining breeds of larger mature size reach puberty at a greater weight than that observed for slower-gaining breeds of smaller size. Breeds historically selected for milk

Table **31-1**

Scrotal Circumference (SC) by Breed in Bulls at 1 Year of Age*

Breed	Number of Bulls	Weighted Mean SC (cm)
Simmental	1246	34.7
Brown Swiss	260	33.8
Gelbvieh	261	33.9
Pinzgauer	144	33.7
Charolais	1887	32.5
Limousin	345	29.8
Blonde d'Aquitaine	15	29.7
Salers	45	29.5
Tarentais	14	32.0
Maine Anjou	64	32.2
Hereford	1567	31.9
Angus	1051	33.2
Shorthorn	167	31.9
Red Poll	250	32.5
Galloway	132	30.6

*Values corrected to 365 ± 14 days of age.
Data (for 6 studies in the United States and Canada) from Barth AD: Breeding soundness evaluation of bulls. The Western Canadian Association of Bovine Practitioners. Continuing Education, Western College of Veterinary Medicine, Saskatoon, Canada, 2000.

Table **31-2**

Scrotal Circumference (SC) by Breed in Beef Bulls at 2 Years of Age

Breed	Mean SC (cm)
Simmental	38.8
Aberdeen Angus	37.2
Charolais	36.3
Horned Hereford	36.1
Polled Hereford	35.6
Shorthorn	34.9
Limousin	32.2
Texas Longhorn	34.6

Data (for 6 studies in the United States and Canada) from Barth AD: Breeding soundness evaluation of bulls. The Western Canadian Association of Bovine Practitioners. Continuing Education, Western College of Veterinary Medicine, Saskatoon, Canada, 2000.

production (e.g., Braunvieh, Gelbvieh, Red Poll, Pinzgauer, Simmental) reach puberty at significantly younger ages than those typical for breeds not selected for milk production (e.g., Charolais, Limousin, Hereford). Great differences are recognized between breeds of bulls in average testicular size at any given age. In general, the large milk-producing beef breeds have an earlier onset of puberty and develop larger testicles at an earlier age and at maturity than smaller breeds of cattle that have lower milk production. Double-muscled breeds such as Piedmonte, Belgian Blue, Parthenaise, Blonde d'Aquataine, and Limousin have a later onset of puberty and smaller average testis size at puberty and at maturity. Few breeders have made an effort to select for larger testis size in these latter breeds; consequently, the breed averages for SC are small. Some breeders, however, have capitalized on the high heritability of testicle size and have made remarkable progress selecting for that trait. Breeders and producers alike are encouraged to select for average or above-average SC, just as they would select for calving ease, weaning weight, or yearling weight. SC data for yearling bulls and 2-year-old bulls are available for many of the common beef breeds (Tables 31-1 and 31-2).

Technique of scrotal circumference measurement. After the testes and epididymides are palpated to ensure that they are normal, the testes are positioned firmly into the lower part of the scrotum so that they are side by side, and scrotal wrinkles that may inflate the measurement are eliminated. This maneuver is particularly important in cool temperatures if accurate results are to be obtained. The testes are held down within the scrotum by placement of the fingers and thumb at the sides of the scrotal neck; however, if the testes are forced down too strongly, softer testes will compress and expand horizontally, resulting in an inflated measurement. The examiner's fingers or thumb must not be placed between the testes, because this will force them apart, also falsely increasing the measurement. A looped tape measure is placed around the greatest diameter of the testes and pulled snugly so that the tape is firmly in contact with the entire circumference. Measurements must be done carefully and repeated to ensure accuracy and repeatability.

Sources of error and reduced repeatability in scrotal circumference measurements. The amount of tension applied by different practitioners can be a source of error in spite of the fact that normal testicular tissue compresses only slightly. The development of spring-scale attachments on scrotal tapes should reduce the variability in SC measurements between veterinarians. When large groups of sale bulls are measured under similar conditions by the same person, the use of a spring-scale scrotal tape is likely to increase producer confidence in SC measurements. Nevertheless, bull weight changes, differences in ambient temperatures at the time of SC measurement, and other factors will still cause variation in reported results on the same bull. For example, in cool temperatures, even if the testes have been carefully pushed down into the scrotum, a larger measurement probably will be obtained than when the scrotum is fully relaxed in warm temperatures.

It appears that fat bulls consistently have larger SC measurements than those obtained after a period of weight loss. In one experiment, 251 bulls of Horned Hereford, Polled Hereford, Aberdeen Angus, and Charolais breeds were evaluated at the time of sale and again just before the breeding season approximately 2 months later. Horned Hereford, Polled Hereford, Angus, and Charolais bulls lost weight over the interval from sale to prebreeding at a mean rate of 2.02, 1.82, 1.71, and 0.94 kg per day, respectively. The rates of loss in backfat and in SC corresponded with the rates of loss in body weight. The loss in SC over the interval from sale to prebreeding was 2.31, 1.95, 2.16, and 1.83 cm for Horned Hereford, Polled Hereford, Aberdeen Angus, and Charolais bulls, respectively.

The cause of the loss in SC most likely was due to weight loss between March sales and May breeding soundness evaluations. The cooler sale barn temperatures in March, however, also may have contributed to the differences in SC measurements.

Changes in testicular tone due to testicular degeneration can occur very rapidly. Within a period of 2 weeks it is quite possible for SC to decrease 2 to 4 cm or even more in severe cases. Subsequently, regeneration and regaining 2 to 4 cm in SC may be observed over 1 to 3 months. These changes will be reflected by changes in testicular tone and semen quality. In such instances, without monitoring of seminal quality, the differences in measurement may be erroneously assumed to be due to differences in technique among veterinarians.

Transrectal Internal Examination

The main focus of the transrectal internal examination is on the accessory sex glands and the inguinal rings. The accessory sex glands of the bull include the prostate, bulbourethral, and vesicular glands. The vesicular glands are lobular structures 8 to 15 cm long, 3 to 5 cm wide, and 1 to 2 cm thick. They lie lateral to the ampullae and neck of the bladder. Congenital defects of the vesicular glands have been reported. These defects usually are unilateral and include aplasia, hypoplasia, cysts, and duplication of the gland. These abnormalities characteristically are associated with anomalies of the mesonephric duct system, which gives rise to the epididymis, ductus deferens, ampullae, and vesicular glands. The bulbourethral glands are embedded in the urethralis muscle caudally near the anal region and are not palpable.

The urethra usually is the first structure palpated. It is a firm tubular structure that usually becomes pulsatile on transrectal palpation as a result of the contractions of the urethral muscle surrounding it. The prostate gland is palpated as a transverse, smooth band surrounding the cranial extremity of the urethra. At this point, the vesicular glands can be palpated craniolateral to the prostate. The vesicular glands vary a great deal in size between bulls. They are smallest in yearling bulls and increase in size with age. They should be uniform in size, lobulated, turgid, and mobile. The ampullae are not very distinct on palpation but can be found by pressing the fingertips over the floor of the pelvis cranial to the prostate gland and moving the fingers back and forth in a lateral direction. The ampullae normally are 10 to 15 cm long and 5 to 8 mm in diameter and can be followed forward to the ductus deferens, which leaves the abdomen through the inguinal rings. Disease of the ampullae often is not clinically detectable. The internal openings of the inguinal canals may be palpable per rectum 15 to 20 cm ventral to the pelvic brim and 5 to 15 cm lateral to the midline. Enlarged inguinal rings are uncommon and are, in most instances, unilateral. A bull with an enlarged inguinal ring will be predisposed to development of a scrotal hernia during breeding. Some evidence suggests that enlarged inguinal rings have a heritable basis.

The most common abnormal finding on rectal palpation is enlargement, excessive firmness, or loss of lobulation of the vesicular glands. Vesicular adenitis occurs in 2% to 4% of yearling and 2-year-old bulls, but the incidence has been reported to be as high as 49%. The incidence in older bulls is less than 1%. Unilateral and bilateral cases of vesicular adenitis have been reported, but unilateral cases are more common. Enlargement with loss of lobulation is the usual finding; long-standing cases, however, may be characterized by adhesions, abscessation, and occasionally, the development of a fistulous tract draining into the rectum, or rarely, the development of peritonitis. In early infections, the glands may not be noticeably enlarged, but neutrophils may be found on examination of semen smears.

In semen smears stained with eosin-nigrosin or eosin–aniline blue, neutrophils with intact membranes appear as white, somewhat irregular bodies with a diameter approximately three times the length of a sperm head. If the neutrophil cytoplasmic membrane is damaged, eosin will enter the cell, staining it pink. To see the characteristic lobulated nucleus of neutrophils, the semen smear must be stained with Wright-Giemsa stain or new methylene blue. Normally, semen smears do not contain any neutrophils. A finding of one or more neutrophils per three microscope fields at 1000× magnification has been suggested as indicative of a significant inflammation somewhere in the reproductive tract. Inflammatory cells usually clump together, however, so large numbers of neutrophils may be found in 1 or 2 fields and none in the next 10 fields. Often, the presence of purulent material in an ejaculate is first indicated by the presence of mucoid streaks and clumps grossly visible on the semen smear. Some caution must be used in interpreting the finding of neutrophils in a semen smear. The source of inflammatory cells may not be the accessory glands or the epididymis. Superficial irritation or injury of the penis and prepuce often results in an abundance of neutrophils on the surface, which may serve as a source of contamination of the semen sample.

The examiner can obtain bacterial pathogens for culture and antibiotic sensitivity testing by collecting semen in a sterile manner with electroejaculation or by passing a long sterile catheter up the urethra and aspirating while massaging the glands. A wide variety of organisms have been isolated from infected vesicular glands; however, in areas in which brucellosis is controlled, *Arcanobacterium pyogenes* is the most common bacterial pathogen found.

SEMEN QUALITY

Methods of Semen Collection

Electroejaculation

Equipment. Currently at least six types of electroejaculators are available on the North American market. Differences between models include how the device is operated and how the bull responds. The kind of electroejaculator used is largely a matter of personal preference.

Rectal probes are available with various diameters and with differences in orientation of the electrodes. Large-diameter probes produce a stronger response to stimuli of a given electrical output than do probes of small

diameter. For most bulls weighing 1200 to 2000 lb (550 to 900 kg), a probe with a diameter of 6.5 to 7.5 cm seems ideal. In larger, older bulls, use of a 9-cm-diameter probe increases the likelihood of adequate stimulus to achieve penile erection and ejaculation. Modern probes have three electrodes spaced about 1 cm apart and are placed completely into the rectum with the electrodes facing ventrally. A U-shaped extension on the back of the probe fits around the tail, ensuring proper orientation of the electrodes during electroejaculation. These probes result in good penile protrusion and electroejaculation with minimal extraneous muscle stimulation. By contrast, the older probes with four electrodes spaced equally around the circumference result in excessive stimulation of the large muscles of the back and hindquarters.

More recently developed probes have three longitudinal ventral electrodes divided into three segments. The segments of the electrodes can be activated separately. The caudalmost segment is used to produce erection and protrusion in all bulls, as well as ejaculation in most yearling bulls. In older bulls, the middle segment and occasionally the cranial segment are used to stimulate ejaculation. The relative effectiveness of segmented probes, or whether they reduce extraneous muscle stimulation or stress in bulls, needs to be evaluated.

Stimulation technique. After examination of the internal organs and inguinal rings by transrectal palpation, the pelvic urethra should be massaged for 30 to 60 seconds in preparation for the collection of semen. The area over the ampulla, prostate, and urethra is massaged by moving the clinician's fingers back and forth in a longitudinal direction. Stimulation will become evident by pulsation of the urethral muscle. In addition, the testes usually will be drawn up, and some clear fluid will drip from the prepuce. Sexual stimulation of the bull by massage seems to facilitate semen collection by electroejaculation. The rectum must be evacuated of feces, and care must be taken to prevent air from entering the rectum. Insertion of the rectal probe is facilitated by lubrication of the probe and rapid massage of the anal region to promote relaxation of the anal sphincter.

The amount of electrical stimulation should be gauged at all times by the response of the bull, rather than by the reading of the meter on the machine. The first stimulus should be applied slowly until the bull gives a minimal response. Each successive stimulus should then be increased slightly in intensity. Stimuli should last 1 or 2 seconds and then be discontinued for about 0.5 second before the next is applied. When the fluid emitted begins to get cloudy, the collection cone is placed over the penis and a sample is collected. If the bull fails to protrude the penis, an assistant should push on the sigmoid flexure behind the scrotum. The operator should be ready with a gauze sponge to grasp the penis when it protrudes. Stimulation usually is continued until 2 to 5 ml of semen is collected.

Bulls usually emit semen without a great deal of stimulation; however, if maximal stimulation has been reached without ejaculation, a total of four or five maximal stimuli should then be given, followed by a 1- to 2-minute rest. The penis should be grasped with a gauze sponge and held during the rest period, because once the penis is retracted, it often will not be protruded during a second attempt at semen collection. Often semen is emitted as the bull begins to relax during the rest period, and the operator must be ready to collect it. As soon as the bull has had a short rest, a second attempt can be made to collect semen. In most instances, semen will be ejaculated on the first few stimuli after the rest period. A similar rest period can be given to a bull that ejaculated easily on the first attempt when a second sample is desired. Frequently, the second sample will be more concentrated than the first.

Difficulty in obtaining semen samples by electroejaculation may be due to several factors. Insufficient electrical stimulus may be due to presence of an excessive amount of air or feces in the rectum, inadequate probe size, a buildup of varnish on the probe electrodes, or weak batteries in the electroejaculator. Electroejaculation seems to be more difficult in fractious, frightened bulls; however, tranquilization of bulls with acepromazine, but not xylazine, is likely to increase the difficulty in obtaining a semen sample. Bulls that have ejaculated repeatedly in the previous 24 hours (e.g., bulls in breeding use) often are difficult to stimulate to ejaculation by this method and produce samples of low sperm density.

Semen cannot be collected by electroejaculation from 1% to 2% of normally fertile bulls despite every effort. Several alternatives to electroejaculation that may be considered are semen collection by massage, use of an artificial vagina (AV), and retrieval of semen from the vagina immediately after breeding.

Massage

Semen can be collected from many bulls by transrectal massage. Sexually rested, quiet mature bulls that have been handled calmly are good candidates for this technique. The hand of the operator is introduced into the rectum, and after the inguinal rings and accessory sex glands have been examined, a longitudinal back-and-forth massage is applied mainly over the ampullae and prostate and periodically over the urethra. Stimulation of the vesicular glands by this method appears to be of minor importance. Massage should be continued until a semen sample is collected, but if no semen is collected within 2 to 3 minutes, usually further massage will be unsuccessful.

Semen collection by transrectal massage carries some disadvantages and may not be practical in all situations. Some of the disadvantages include rectal mucosa irritation, incomplete penile protrusion, the necessity of a second person to collect the semen sample, and difficulty in stimulating excited or fractious bulls. Also, the number of bulls in which this method can be applied at one session is limited, because the procedure is very tiring for the person doing the massage.

Use of an Artificial Vagina

Semen collection with an AV is performed almost exclusively in artificial insemination centers. The method is not used routinely in breeding soundness evaluations but may be considered in bulls that have normal testes and epididymides but produce poor semen samples or no semen at all by electroejaculation. The AV method of

collection sometimes is preferred in prepurchase examinations by artificial insemination centers and by personnel from foreign countries interested in importing bulls.

Bulls considered for collection by AV should be halter-broken and nose ring–trained. The mount animal should be restrained in a short-sided breeding chute. Bulls that are not accustomed to serving an artificial vagina may need a female in estrus in order for the procedure to be successful. The female may need to be sedated (xylazine, 0.03 mg/kg IV) to facilitate collection. The AV is prepared by filling the water jacket with water hot enough to result in a final AV temperature of 42° to 50° C. Air can be added to increase the pressure until the AV liner bulges out of the end slightly. Finally, the AV is lubricated with a sterile nonspermicidal gel. When the bull mounts, the collector must be ready to step in immediately and direct the penis to the opening of the AV. The penis is never handled directly; rather, the operator directs the penis by grasping it through the sheath. The AV is not shoved onto the penis; the bull will make seeking motions and must be allowed to thrust into the AV. The thrust may be extremely vigorous, so that an unwary collector may be knocked off balance or the AV knocked out of the hand.

Collection of semen with an AV from untrained range bulls by conventional means often is unsuccessful. In my experience, however, approximately 85% of untrained range bulls will mount a restrained estrous female and serve an AV if the person handling the AV cannot be seen by the bull. This can be accomplished by fixing a canvas tarp beside the mount cow as a blind. In addition, a triangular piece of tarp must be fashioned to extend perpendicularly across the cow's rump to prevent the bull from seeing behind during mounting.

An alternative to the conventional AV is the recently designed intravaginal AV. Nearly all bulls that normally will serve a cow will readily serve an intravaginal AV.

Finally, a bull may be allowed to breed an estrual female whose vagina has been flushed with saline to remove mucus. After the female is bred, it usually is easy to aspirate a small amount of semen from the cranial vagina.

Evaluation of Semen Quality

Semen Density and Volume

Unlike semen evaluation in the stallion, boar, and dog, in which the total number of spermatozoa in an ejaculate often is used to estimate potential sperm output, the capacity to produce sperm in bulls is determined by measurement of SC. With a regular schedule of semen collection using an AV, as in artificial insemination centers, ejaculate volume and density (sperm output) provide meaningful information about a bull's capacity to produce sperm. With electroejaculation of bulls, however, many factors may affect the volume and density of semen samples, making these characteristics unreliable for evaluation of semen quality. Nevertheless, when several milliliters of concentrated semen are collected with reasonable ease, it provides some assurance that the bull is capable of producing good ejaculates.

The seminal traits of sperm density and motility (described next) usually are described as very good (VG), good (G), fair (F), or poor (P). These descriptors have been in use for longer than 30 years and have specific meanings. The following descriptors are used for sperm density:

VG = creamy, grainy semen with 750 million to 1 billion or more sperm per milliliter
G = milk-like semen with sperm counts of 400 million to 750 million/ml
F = skim milk–like semen with sperm counts of 250 to 400 million/ml
P = translucent semen with sperm counts of less than 250 million/ml

Sperm Motility

Spermatozoa are in a quiescent state within the cauda epididymidis. In order to be motile, spermatozoa are dependent on a normal pH, warm temperature, an osmotically balanced medium, and adequate concentrations of ions and nutrients in seminal fluid.

It is very easy to inhibit motility by contamination of the semen sample or improper handling procedures. For example, glassware used in motility estimates may be contaminated by soap residue from washing, or small amounts of soap may be splashed onto slides from nearby sinks, and chemicals present on the fingers may be transferred to slides during handling. Semen samples may become contaminated by urine during electroejaculation or may contain pus. Cold or hot test tubes, glass slides, or microscope stages, as well as rapid drying or cooling of a drop of semen on a slide exposed to wind, will quickly destroy sperm motility. Seminal fluids do not maintain sperm viability for very long, and spermatozoa quickly lose their vigor of forward progression in semen samples that are not examined soon after collection. Repeated ejaculations may result in improved sperm motility in a few bulls that have not ejaculated for a long time.

Gross motility. Gross motility is determined from a 5- to 10-mm-diameter, non-coverslipped drop of semen placed on a warm slide under brightfield microscopy at 40× to 125× magnification. To increase contrast, the condenser diaphragm should be closed, or in microscopes with no diaphragm, the condenser should be lowered. The following descriptors are used for gross motility:

VG = rapid dark swirls
G = slower swirls and eddies
F = no swirls, but prominent individual cell motion
P = little or no individual cell motion

Mass motion is dependent on three factors: concentration, percentage of progressively motile cells, and the speed of progression of spermatozoa. When any one of these factors is depressed, the rapid swirling expected will be severely depressed or eliminated. Semen with fair concentration may have 80% rapidly progressively motile sperm but show no wave motion, whereas highly concentrated semen may have only 50% motile sperm and still show some slow wave motion. On the other hand, semen with very good concentration and a high percentage of progressively motile sperm may have little or

no wave motion if the speed of sperm progression has been diminished by cold temperature or a prolonged interval from collection to examination. Therefore, gross motility must be carefully interpreted. Wave motion is expected when semen samples have good concentration. If wave motion is present, no further examination for motility usually is necessary. If it is absent, a wet mount must be examined for individual progressive motility. When semen samples are dilute, wave motion is not expected, and wet mounts must be prepared for evaluation of individual motility.

Individual motility. New, perfectly clean, warm slides and coverslips are necessary in the preparation of wet mounts. Dust particles on the slide will prevent even fluid dispersion under the coverslip. With well-made wet mounts, semen dilution is often not required to make an estimate of the percentage of progressively motile sperm. A drop of semen 3 to 4 mm in diameter usually is large enough for the fluid to spread barely to the edges of the coverslip, forming a very thin layer of fluid in which individual spermatozoa are all within the same focal plane. Wet mounts should be examined at 200× to 500× magnification, preferably under phase-contrast microscopy. It is difficult to see individual sperm motion under brightfield microscopy even with the diaphragm closed or the condenser lowered. Many modern student microscopes that find their way into clinical practice can be fitted with phase contrast adaptors for 400× magnification at a cost of $600 to $800. For individual progressive motility, the following descriptors are used:

VG = 80% to 100% motile
G = 60% to 79% motile
F = 40% to 59% motile
P = less than 40% motile

Observation of individual motility and estimation of the percentage of progressively motile sperm will provide information about sperm membrane integrity, as well as the morphologic integrity of spermatozoa. For example, if a high percentage of spermatozoa has normal progressive motility, then a finding of a high percentage of bent midpieces, or dead sperm will indicate mishandling of the semen, rather than an inherent abnormality. Similarly, poor motility is consistent with abnormal sperm morphology and dead spermatozoa. If inconsistencies in motility and morphology observations arise, the cause should be established. It may be necessary to repeat the semen collection and evaluation procedure.

Sperm Morphology

Preparation of semen smears. Spermatozoa are translucent and virtually invisible on brightfield microscopy. Visualization therefore requires staining of spermatozoa or provision of a dark background for highlighting the sperm. For example, rose bengal stains all spermatozoa pink against a clear background, whereas India ink provides a dark background and all spermatozoa remain unstained and appear white. The so-called live-dead stains use nigrosin or aniline blue to provide background and eosin as a vital stain. Eosin penetrates damaged cell membranes, staining injured or nonviable sperm pink (dead), while viable sperm repel eosin and appear white (alive). When normal semen has been handled properly and staining is carried out correctly, the percent of sperm staining alive is highly correlated with individual progressive motility. One-step stains, such as eosin-nigrosin, that can be mixed with semen on the slide are preferable because everything that is in the semen will be visible. Staining techniques that require several steps, including washing, are more damaging to spermatozoa, with greater potential for some elements in semen to be washed off, resulting in less reliable differential counts.

Commonly used formulations of eosin-nigrosin and eosin–aniline blue stains are very hypotonic, having an osmolality of 90 to 100. Some buffers cannot be added to these stains, because the salts precipitate and obscure the spermatozoa when the smears are made and dried. Hypotonic shock is easily induced in living spermatozoa by these stains; risk of such injury must be minimized by drying smears as quickly as possible. Fast drying is possible only when glass slides are warm. Blowing air over the smear speeds up drying. The artifact of hypotonic shock is easily recognized as a whipped-around appearance of the terminal portion of the sperm principal piece. Less commonly, the midpiece bends distally. Because the distal droplet usually would be shed before the occurrence of hypotonic shock, no droplet material would be included in the bend. From 5% to 30% of spermatozoa within the cauda epididymidis do not have a distal droplet, however, so the finding of a distal midpiece reflex without a trapped droplet does not necessarily imply hypotonic shock. Observation of sperm motion under a coverslip is very useful to distinguish between tail defects caused by epididymal malfunction and artifactual causes of bent tails.

The addition of glucose or TES-Tris to eosin-nigrosin, raising the osmolality to 266 mOsm/kg, was shown to decrease membrane damage due to staining in boar sperm. This formulation may be useful in preventing hypotonic shock in bull semen.

In preparation of semen smears, a 5- to 6-mm droplet of live-dead stain should be placed at one end of a warm slide. A 3- to 5-mm droplet of semen is then placed beside the stain to control its size before mixing with the stain. The size of the semen droplet depends on the sperm cell concentration. After the stain and semen are mixed on the slide, the mixture is spread slowly from one end of the slide to the other by drawing along the drop with the edge of another slide or with the side of a wooden applicator stick.

Differential counts of sperm morphology must be done using oil immersion plates at 1000× to 1250× magnification. Lower magnifications simply do not reveal a multitude of possible serious defects. With only a few sperm abnormalities in an individual sample, counting 100 sperm is sufficient. When many abnormalities are present, however, a count of 300 or more sperm will provide a more accurate differential count.

Classification of sperm abnormalities. Although the primary and secondary sperm defect classification system has been widely accepted, this system of classification often means different things to different practitioners. By definition, a *primary defect* is one that originates within

the testis during spermatogenesis. A *secondary defect* is one that originates within the epididymis. All head defects such as knobbed acrosomes, pyriform heads, microcephalic sperm, and nuclear vacuoles are therefore primary defects. Most tail defects, including the Dag defect, mitochondrial sheath defects, and coiled principal pieces also have their origins in spermatogenesis and are primary defects. The **Dag defect**, which results from abnormal development of the axoneme and mitochondrial sheath, expresses itself as a shattered midpiece within the epididymis and has therefore been called secondary by some investigators. By definition of origin, however, the Dag defect would be a primary defect.

Some defects can be either primary or secondary. Proximal droplets may be the result of either a disturbance of spermatogenesis (primary) or a disturbance of epididymal function (secondary). Similarly, detached heads may be due to a defect of the basal plate, which connects the sperm head to the midpiece capitellum (primary), or it may be due to abnormal epididymal function (secondary). The **distal midpiece reflex defect**, which develops in the epididymis, appears not to have its basis in spermatogenesis and would thus be a secondary defect. Sperm with distal droplets have never been reported to be associated with infertility and probably should not be classified as defective. This sperm aberration does not appear to originate in the testis or epididymis but rather (when found in large numbers) results from a lack of a hemolytic factor in seminal fluid.

An important point is that the definition for primary and secondary sperm defects denotes the origin and not the severity of a defect. Furthermore, because adverse conditions that cause both types of defects can affect epididymal function and spermatogenesis simultaneously, primary and secondary defects are equally important as indicators of a disturbance of reproductive function. The first sperm defects to appear in semen after the onset of a disturbance of spermatogenesis are epididymal in origin, and these are followed by primary defects. Therefore, the concept that primary defects have a more important effect than that of secondary defects on bull breeding soundness may be questionable in many instances.

Blom attempted to improve the method of classifying sperm defects with the "major" and "minor" defect system.[8] In this system, major defects are those that have been associated with infertility. Minor defects are those sperm aberrations that were not associated with infertility at the time the system was devised. Some defects considered to be minor involved obvious structural abnormalities, and fertilization or embryonic development probably would be impossible using affected sperm. Some examples are small heads, giant heads, and simple bent tails (distal midpiece reflexes). At the time the major-minor system was proposed, these defects had never been identified in large numbers in semen, even though many bulls had been examined at Blom's laboratory. Therefore, these defects were considered to be minor. As Blom predicted, however, with the development of new information, flaws in the system became evident. For example, distal midpiece reflexes that initially were considered to be minor may be found as a heritable defect in Jersey bulls affecting up to 100% of spermatozoa.

Accordingly, it probably would be appropriate to cease classifying sperm defects. Instead, the prevalent sperm defects should be considered in regard to the current understanding of their significance. Because some sperm defects seem more important to fertility than others, a differential count of the defects should be done. In general, a minimum number of normal, live spermatozoa must populate the oviduct to optimize the possibility of a sperm cell's finding the ovum, penetrating the zona pellucida, fertilizing the ovum, and initiating and sustaining embryonic development. Sperm numbers in the oviduct are affected by sperm transport mechanisms within the female reproductive tract and by the dosage of spermatozoa delivered. Once in the oviduct, spermatozoa require normal membrane receptors to bind to the zona pellucida, a normal acrosome and tail in order to penetrate the zona, and a normal nucleus for fertilization.

A great deal remains to be learned about the various sperm abnormalities in regard to sperm transport through the cervix and the uterotubal junction. Progressive sperm motility evidently is essential for transport through the cervix. Once sperm have passed through the cervix, uterine transport mechanisms move both defective and normal sperm into the oviduct; however, the uterotubal junction also may filter a proportion of sperm abnormalities.

It has been shown that spermatozoa with nuclear vacuoles—the diadem defect—are transported normally in the female reproductive tract and that they may penetrate the ovum. This is a very important finding because zona penetration would be expected to result in the cortical reaction, rendering the zona pellucida impervious to other spermatozoa. Increasing sperm numbers in the oviduct would not improve the chances of normal fertility. For example, if a semen sample had 20% diadem defects, it would make no difference to fertility whether 100, 1000, or 10,000 sperm were present in the oviduct; the chance of a vacuolated sperm cell's penetrating the zona pellucida and initiating a zona reaction would always be 20%. On the other hand, spermatozoa unable to penetrate the zona pellucida, such as those with knobbed acrosomes or bent tails, would be unable to induce a zona reaction and thus not prevent normal sperm from fertilizing the ovum. In such instances, increasing the insemination dosage would increase the probability of fertilization. This has led to the concept of **compensable** (by dose) versus **noncompensable defects**.[9]

Abnormal sperm that are not transported to the oviduct, and those that are transported but are not capable of penetrating the zona pellucida may be compensated for by increasing sperm dosage (compensable defects). Abnormal sperm that are not filtered and that are capable of penetrating the zona pellucida, causing a zona reaction, cannot be compensated for by increasing the sperm dosage (noncompensable defects). Although this may be an oversimplified view of aberrant sperm function, it is helpful for the clinician to think of defective spermatozoa in this way when trying to determine whether a bull's fertility may be affected by a given level of specified sperm defects. Recent in vitro fertilization

studies have shown that although diadem sperm will penetrate the zona pellucida, they are less efficient in doing so than sperm without the defect. Therefore, the diadem defect potentially may be partially compensable. Normal sperm coexisting in semen with a high percentage of proximal droplets were found to be less efficient in attaching to, and penetrating, the zona pellucida than normal sperm from unaffected bulls. Proximal droplet sperm, then, cannot be compensated for by simply increasing dose size. The most common abnormal morphologic forms are depicted in Figure 31-1. More information about abnormal sperm morphology may be found in other sources.[10,11]

Tolerance levels of sperm abnormalities. Many experiments have shown a correlation between sperm defects and infertility. Because of the wide variety of factors that affect herd fertility with both natural breeding and artificial insemination, however, few studies have had sufficient sensitivity to establish the tolerable levels of various sperm defects that would be compatible with good fertility. The tolerable levels that are widely accepted today were established by early workers and have been confirmed by more recent work. Generally, the upper limit for sperm nuclear (head) defects is in the range of 15% to 20%, whereas acrosomal and tail defects in up to 25% of sperm may be tolerated. At least 70% of spermatozoa should be normal.

Interpretation of sperm morphology counts. For many veterinarians, the evaluation of sperm morphology is viewed only as a means to determine whether a bull is capable of causing high conception rates. In fact, however, spermatozoa are in a sense a sample of testicular tissue and as such provide information about the health of the seminiferous epithelium and the epididymis. An abnormal spermiogram, when interpreted in light of the bull's history and findings on the physical examination, may suggest the cause of abnormal testicular and epididymal function, a possible course of treatment, and a prognosis for recovery to normal sperm production.

An understanding of normal and abnormal spermatogenesis greatly facilitates the interpretation of a spermiogram. Abnormal spermatogenesis has many possible causes; in most instances the disturbance can be

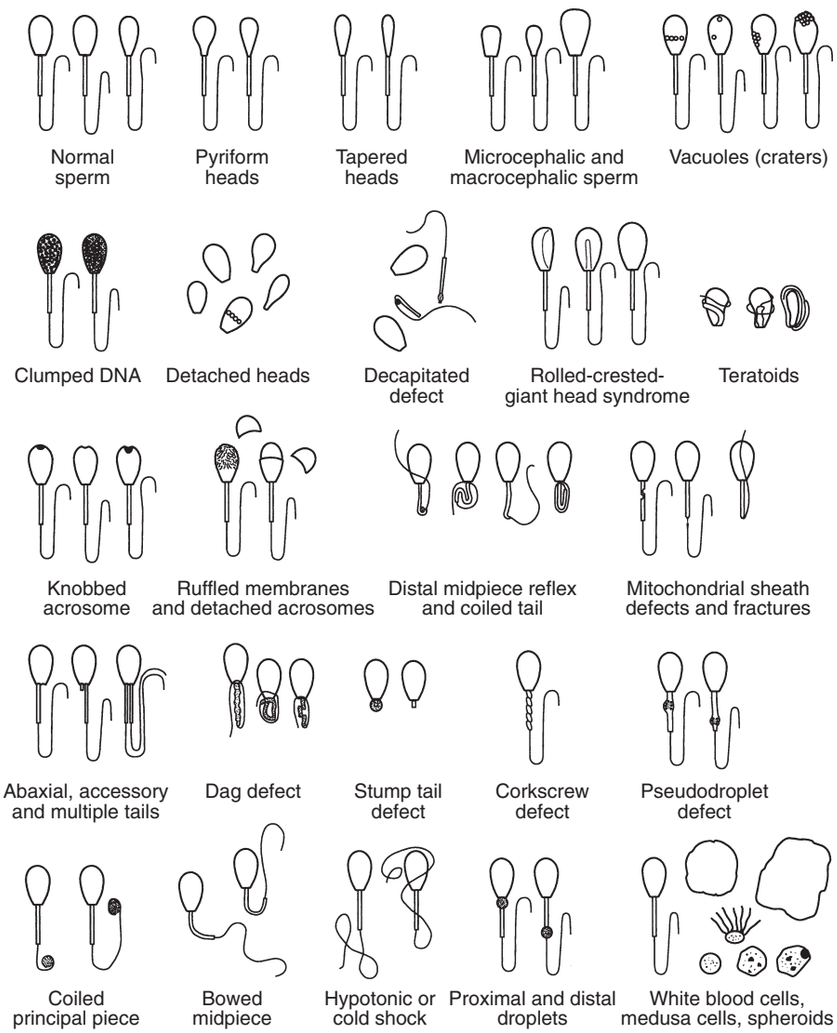

Fig. 31-1 Common abnormal morphologic forms of spermatozoa.

categorized as heat related or stress related. Other, less common causes include genetic, toxic, and perhaps nutritional deficiency. An increase in the temperature of the testes of a bull by 0.5° to 1.0°C maintained for a few days is sufficient to cause a noticeable disturbance of spermatogenesis. It appears that the mechanism of heat damage is through tissue hypoxia. Normally, because of the peculiar anatomy of the blood supply to the testis, the tissue operates on the brink of hypoxia. Raising testicular temperature increases the metabolic activity without a corresponding increase in oxygen supply, and tissue hypoxia ensues.

Normal functions of the epididymis and the seminiferous epithelium are dependent on very high levels of testosterone. Some evidence suggests that hypoxia (heat) also results in reduced testosterone available to the developing germ cells. For example, the Sertoli cells concentrate testosterone by producing androgen-binding protein (ABP). Impaired Sertoli cell function or Leydig cell function may result in reduced testosterone levels.

Stress due to illness, injury, severe and prolonged low ambient temperatures, starvation, transport and environmental changes (e.g., show circuit stress), and pain (e.g., sole abscesses, laminitis, arthritis, dehorning) affects testicular function through an endocrine mechanism. Stress results in excess adrenal cortisol production, which in turn decreases pituitary luteinizing hormone secretion, resulting in reduced testosterone production by the Leydig cells.

Some cells are less susceptible to damage than others; the spermatogonia and Sertoli cells are the least susceptible. The cells in meiosis are quite sensitive to insults and subsequently degenerate, leaving empty spaces in the germinal layer, with reduced sperm output consequent to the decrease in number of cells. The spermatids also are quite sensitive and respond to insults by forming abnormal spermatozoa. For example, a disturbance of spermatogenesis may result in poor condensation of the nuclear chromatin, vacuolation, abnormal head shapes, malalignment of mitochondria in the midpiece, or lack of cementum between fibers of the sperm tail.

Severe disturbances of spermatogenesis result in sloughing of cell layers into the tubular lumen, leaving only Sertoli cells and spermatogonia. This is known as **degeneration.** The empty tubules lose turgidity; thus, testes that have undergone degeneration become softer and smaller. After the insult to spermatogenesis is removed, the spermatogonia repopulate the tubules, the testes may return to normal size and consistency, and normal quantities and quality of semen can be produced. When there is a disturbance of spermatogenesis, usually not all tubules are affected to the same degree. Within a histologic section, completely normal tubules and tubules with cellular damage can be seen; therefore, ejaculates usually have varying proportions of normal and abnormal sperm.

Although heat and stress apparently affect testicular function through different mechanisms, no difference in the types of resulting sperm abnormalities have been observed between the two. A more detailed discussion of spermatogenesis and sperm abnormalities can be found in other sources.[10,11]

BREEDING SOUNDNESS CLASSIFICATION

The four categories of breeding soundness are **satisfactory, questionable, decision deferred,** and **unsatisfactory.** Bulls classified as satisfactory potential breeders are those that have met the minimum requirements for physical soundness and semen quality. Bulls that have unknown sex drive and mating ability may be classified as satisfactory, but documentation of the animal's breeding soundness must mention that this aspect needs to be examined by the producer. The questionable category is for bulls likely to perform adequately in mating but with below-normal fertility, or with an undesirable trait with the potential for genetic transmission to offspring. Some examples follow:

- Bulls with scrotal circumference below the recommended minimum, but with satisfactory semen quality.
- Bulls with conformational abnormalities. A bull with corkscrew claws may be highly fertile, but because the claw defect is heritable, selection of offspring (replacement heifers) from such a bull would eventually lead to foot problems in the cow herd.
- Bulls with high percentages of compensable sperm defects, or a little less than 70% normal sperm at the beginning of the breeding season. Although such bulls may recover normal sperm production at a later date and therefore could be assigned to the category of decision deferred, they would be questionable for immediate use.
- Bulls with a hereditary sperm defect. A defect such as the knobbed acrosome defect affecting 25% of sperm may not reduce fertility significantly. Because the affected bull is likely to carry a recessive gene for the defect, however, it is questionable whether he should be used by a purebred breeder.
- Bulls with an abnormal accumulation of senescent sperm. Such bulls probably would fail to impregnate females for approximately the first week of the breeding season. After repeated ejaculations, however, the accumulation of senescent sperm from the ampullae and cauda epididymidis would be replaced by "new" spermatozoa, and fertility would be expected to become normal.

The decision deferred category is intended primarily for pubertal bulls with poor semen quality. Poor semen quality is expected in pubertal bulls; however, whether normal semen quality will be produced at sexual maturity is unknown. Therefore, the decision on breeding potential should be deferred. The decision deferred category also may be appropriate for mature bulls that have suffered a recent disturbance of spermatogenesis from which recovery is expected before the onset of the breeding season. For example, bulls with mild scrotal frostbite often have poor semen quality in early March, but recovery usually occurs before the May/June breeding seasons.

The unsatisfactory classification should be used for bulls whose use is expected to result in poor fertility in

the cow herd during the impending breeding season. If recovery to normal fertility is possible, this can be stated in the appropriate document.

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CHAPTER 32

Diseases of the Reproductive System of the Bull

FRED M. HOPKINS

Conception failure may be associated with female factors, male factors, or both. Too often, it is assumed that infertility is a female problem, and few bulls are examined for breeding soundness before the breeding season. Most bulls are examined because of reproductive failure discovered during or after the breeding season. Estimates of the proportion of bulls having significant reproductive problems range from 5% to 25%. Very often the most cost-effective way of dealing with reproductive diseases of the bull is culling and replacement.

COMMON PENILE PROBLEMS

The lesions of **penile fibropapillomatosis** are warts. Fibropapillomas are the most common mass seen on the bull's penis and have a viral etiology. The causative virus is believed to gain entry into the preputial epithelium through abrasions of the penis associated with mating activity among bulls. This problem usually is seen only in young bulls and is not associated with warts on other parts of the body.

Clinical signs may not be evident in penile fibropapillomatosis, and the problem may be recognized only during a breeding soundness evaluation. Bleeding from the preputial opening may be seen, along with reluctance to mate. Phimosis or paraphimosis may result from the presence of larger masses.

Penile fibropapillomas may occur as single or multiple masses. The mass usually is pedunculated, but sessile lesions with a diffuse distribution also are possible.

Fibropapillomas sometimes will regress without treatment. Surgical removal of pedunculated masses is straightforward. Commercial wart vaccine has been used in treatment, with variable results. Commercial autogenous wart vaccine may be more useful in preventing recurrence of the problem, which may occur in up to one third of affected animals.

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Fibropapillomas sometimes will regress without treatment. Surgical removal of pedunculated masses is straightforward. Commercial wart vaccine has been used in treatment, with variable results. Commercial autogenous wart vaccine may be more useful in preventing recurrence of the problem, which may occur in up to one third of affected animals.

Persistent penile frenulum is a congenital band of tissue extending from the median raphe of the prepuce to the ventral side of the penis near the glans. Penile erection and extension in affected animals results in a ventral bowing of the penis. In bulls with longer prepuces, the prepuce may be pulled over the glans penis by the band. In either case, successful intromission is unlikely. This condition is believed to be heritable, and affected animals should be used only as terminal sires.

Surgical correction of persistent penile frenulum requires that the penis be extended and secured. Local anesthetic injection is given at each end of the band. Blood vessels and the band are ligated, and the band is transected and removed. Healing occurs rapidly.

Inflammation of the penis and prepuce occurs occasionally in younger bulls. Pain resulting from this inflammation may be severe enough that the affected bull mates less often or not at all. Most frequently, this condition is associated with abrasions occurring at mating or infection with bovine herpesvirus type 1 (BHV-1) (i.e., infectious bovine rhinotracheitis–bovine virus diarrhea [IBR-BVD]). BHV-1 infection causes small areas of focal necrosis, resulting in 1- to 3-mm-diameter ulcers. Inflammation of the penis and prepuce may be severe enough to produce adhesions, with limitation of future fertility. In most cases, however, the problem is self-limited and will resolve after 2 weeks of sexual rest.

Hair rings encircling the penis may be seen, especially in younger bulls that are housed in groups. It is believed that these hair rings result from mating activity during which the penis is rubbed across loose body hair. Eventually, a ring of hair may accumulate on the penis, resulting in discomfort, necrosis, and occasionally, urethral fistula or penile amputation. Removal of the hair ring and local wound therapy should result in the resolution of reversible pathologic changes.

DISEASES OF ACCESSORY SEX GLANDS

The most commonly recognized disease of the internal genitalia of the bulls is **vesiculitis.** Inflammation of the vesicular glands most frequently is diagnosed in bulls younger than 2 years of age or older than 9 years. The incidence of vesiculitis usually is low but can be quite high in some groups of young bulls. Inflammation of other parts of the reproductive system often is seen with vesiculitis. The effect of vesiculitis on fertility is variable. Although it is not uncommon for no infectious agent to be identified as the cause of vesiculitis, a number of bacteria and viruses have been associated with this problem. *Brucella abortus, Arcanobacter pyogenes, Haemophilus somnus,* and others have been described as bacterial causes of vesiculitis. The IBR virus and enteroviruses have been incriminated.

The pathogenesis of vesiculitis has not been clearly established. A number of risk factors for this condition have been reported. Young bulls that are housed in contained groups and fed high-energy rations seem to be more susceptible. Slaughterhouse studies have shown an association between vesiculitis and other infectious diseases such as pneumonia, liver abscesses, and navel ill.

Most affected bulls do not show visible signs of vesiculitis. Occasionally, signs of abdominal pain or rear leg lameness may be noted. More often, this disease is diagnosed during a routine breeding soundness evaluation or during examination of the bull as a part of a herd infertility investigation.

Considerable variation in vesicular gland size, consistency, and texture is normal in bulls. Consequently, the interpretation of findings on rectal examination may be difficult. Inflamed vesicular glands are enlarged, painful, and firm. Loss of lobulations may be noted. The vesicular glands may be adherent to adjacent structures. Abscessation can occur. Changes may be unilateral or bilateral. The ejaculate of affected animals may be darker than normal in color and contain clumps. Microscopic examination characteristically reveals an increased number of polymorphonuclear cells. These cells cannot be identified using the usual eosin-nigrosin sperm stain, and a white blood cell stain is necessary. Sperm motility generally is lower in affected bulls.

Bulls older than 9 years of age seldom recover from vesiculitis. Usually, these bulls are culled. Bulls younger than 2 years often recover within 6 months with no treatment at all. Medical treatment of this condition is based on long-term antibiotic therapy. The culture of routinely obtained semen samples is not useful, because gross contamination is the rule. A procedure for the collection of fluid for culture has been described. After cleansing of the ventral abdomen, the penis of a suitably tranquilized bull is extended and secured. A catheter is passed up the urethra and fluid is collected during rectal massage. Even with favorable laboratory results, treatment with appropriate antibiotics often is disappointing. Treated bulls often improve during treatment, only to relapse after the course of therapy is complete. Macrolide antibiotics and flunixin often have been recommended for cases of seminal vesiculitis. Surgical removal of diseased glands is difficult, but a newly described ventral pararectal approach shows promise. Culling of affected bulls probably is the most cost-effective approach. It has been reported that the incidence of vesiculitis in high-risk groups has been reduced by feeding of chlortetracycline.

EPIDIDYMAL DISEASES

Epididymal inflammation, or **epididymitis,** in the bull most commonly is unilateral and involves the tail of the epididymis. Epididymitis may be diagnosed alone but often is seen associated with vesiculitis or orchitis. Infection with bacteria including *A. pyogenes* and *B. abortus* is the most common cause of epididymitis in the bull. Epididymitis often results in infertility secondary to obstruction of the lumen. Thermal injury to the testicle also may result. Inflamed epididymal tails are hot, swollen, and painful early in the course of the disease but chronically become small, hard, and misshapen. Treatment generally is of little value, and most affected animals are culled.

Congenital absence of part or all of the epididymis usually is unilateral in the bull. This segmental aplasia may be hereditary, and affected animals should not be

used for breeding. Segmental aplasia may be accompanied by enlargement of the duct system proximal to the missing area due to sperm stasis. Often, the corresponding vesicular gland or ampulla will be found to be absent.

TESTICULAR DISEASE

Orchitis is infrequently diagnosed in the bull. Generally, only one testicle is affected, but the opposite testicle can quickly undergo degeneration as a result of thermal injury from inflammation. Orchitis generally is considered to be due to infectious causes. Infectious agents may reach the testicles by hematogenous spread from other loci of infection within the body. Infections in other parts of the reproductive or urinary system may extend to involve the testicles. Orchitis may result from wounds that penetrate the scrotal skin. Bacteria, including *B. abortus, Arcanobacter,* and others, are most frequently isolated in cases of orchitis. Certain viruses also may initiate testicular inflammation. The swelling associated with inflammation coupled with an inelastic tunica albuginea leads to pressure necrosis of the testicles. Thrombosis of blood vessels and heat produces degeneration. The testicle rapidly loses its ability to function normally.

The diagnosis of orchitis in the bull is straightforward. Observation of the scrotum shows one testicle larger than the other. On palpation, the scrotal contents will be painful, hot, and edematous. Ultrasound examination and thermography are useful aids in diagnosis. Medical treatments of orchitis may not be completely successful in returning the testicle to normal function. The contralateral testicle is at risk for degenerative changes if resolution of the inflammation is delayed. Antibiotics are of limited value in most cases. Cold water hydrotherapy may be of help. Often, bulls of moderate value are culled. If the bull is more valuable and the rest of his reproductive system is normal, surgical removal of the affected testicle will allow the other testicle to eventually compensate. The remaining testicle probably will produce more than one half of the sperm produced by two.

It is likely that the most common reproductive abnormality of the young bull seen by veterinary practitioners is small-sized testicles. **Testicular hypoplasia** has been defined as testis size smaller than normal for age; one or both testicles may be affected. The definition also presumes that the problem is congenital, with some derangement of germinal cells.

Testicular hypoplasia generally is considered to have a large heritable component. Bulls with small testicles have reduced sperm motility, a lower percent of sperm with normal morphology, and less concentrated semen with fewer total sperm numbers per ejaculate. Affected animals may be subfertile or infertile. Libido is unaffected.

The diagnosis of hypoplastic testicles is based on scrotal circumference measurements and analysis of semen samples. The problem usually is not recognized until after puberty. The Society for Theriogenology has published minimum standards for scrotal circumference in bulls. Scrotal circumference measurements of less than 30 cm, or 32 cm in postpubertal bulls, have been suggested as being diagnostic of testicular hypoplasia in the bull. This problem cannot be corrected, and affected animals should be culled.

Failure of normal testicular descent is referred to as **cryptorchidism.** This problem is relatively uncommon in bulls and usually is unilateral. Because of the possibility that cryptorchidism is inherited, affected animals should not be used for breeding.

Testicular degeneration is an acquired condition in which testicles that were once normal undergo pathologic changes, resulting eventually in small testicular size and abnormal function. Testicular degeneration may affect one or both testicles, and the changes may be temporary or permanent.

A number of factors have been shown to result in testicular degeneration in the bull. Increased temperature of the scrotal contents, even for a relatively short period of time, has been shown to result in testicular degeneration with a higher percentage of abnormal ejaculated sperm or even azoospermia. Fever, high environmental temperature, inflammation of the scrotal skin, and excessive scrotal fat all have been shown to have an adverse effect on testicular function, presumably secondary to increased testicular temperature. Extreme cold resulting in scrotal frostbite is reported to lead to testicular degeneration. Orchitis, testicular trauma, and zeranol implants can result in degeneration of the testicles. Degeneration can result from blockage of parts of the excurrent duct system, such as the epididymis. Bulls seem to undergo testicular degeneration associated with advancing age earlier in life than other animals. Most bulls will undergo changes by 8 to 10 years of age.

Diagnosis of testicular degeneration most often is based on the history, a careful examination of the scrotal contents including scrotal circumference measurements, and semen analysis. Ultrasound examination also has been used.

Treatment is limited to removal of the cause of the degeneration when it can be determined. Re-examination at 45- to 60-day intervals will determine if the changes are temporary or permanent.

OTHER REPRODUCTIVE DISEASES

Penile hematoma occurs as a result of rupture of the tunica albuginea associated with injury at breeding. Affected bulls most often are Polled Hereford and show reluctance to mate, a prolapsed prepuce, and a swelling anterior to the scrotum, which cannot be separated from the penis. Paracentesis should not be used for diagnosis because 60% of the cases progress to abscess formation. Approximately 70% of bulls with hematoma smaller than 20 cm in diameter will recover if given antibiotics and 6 months of sexual rest. About 70% of bulls with hematoma greater than 20 cm in diameter will recover with suturing of the torn tunica albuginea and 3 months of sexual rest. About 50% of bulls with hematomas larger than 20 cm will recover with antibiotics and sexual rest.

Preputial lacerations usually are the result of tearing during breeding and occur most often in Brahma, Brahma-cross, or polled bulls. Affected bulls show reluc-

tance to mate, and on examination, prolapse of the preputial membrane and inflammation with swelling of the cranial one half of the penis and sheath can be seen. Treatment includes local and parenteral antibiotic therapy, along with hydrotherapy and bandaging. The prolapsed portion of the prepuce should be replaced and held in with a pursestring suture as soon as possible. Medical therapy should continue for 10 days to 3 weeks and until infection is controlled and healing is well under way. Surgery (circumcision) is indicated if the prolapsed prepuce cannot be replaced or if the healed laceration produces a stricture so that the bull's penis cannot be extended. Approximately 75% of bulls can be returned to service with some combination of therapy. It is difficult to justify surgical intervention in bulls worth less than $2000.

Lack of libido is difficult to measure or diagnose. Lack of libido may be due to inheritance, inexperience, or back, feet, leg, or joint abnormalities. Lack of libido is not treatable unless a primary medical cause is identified.

CHAPTER 33

Surgical Correction of Abnormalities of the Reproductive Organs of Bulls and Preparation of Teaser Animals

GREGOR MORGAN

Surgical procedures commonly are performed on the reproductive organs of bulls. Although some procedures such as castration are quite simple, when they are performed improperly, the outcome can be costly. Not all surgical failures can be attributed to poor surgical technique, however. Often they are the result of the surgeon's having to perform under less than ideal circumstances (environmental conditions and available facilities, in particular).

In a practice situation, an economic conflict frequently exists between the ideal facility and equipment to perform certain procedures and the number of procedures that can be performed over time. In addition, some procedures are quite difficult, and unless they are performed on a regular basis, familiarity and skill with the technique are not acquired. These factors should be considered when a more difficult procedure is required and referral of the case may be more appropriate. The patients of food animal practitioners have an economic value at slaughter, and this consideration must always be weighed when a costly procedure is considered that may result in loss of the bull's services for the current breeding season. With regard to facilities, most routine procedures performed on the reproductive organs of bulls can be accomplished in a squeeze chute. Other surgical procedures require the animal to be in right lateral or dorsal recumbency, either on a clean ground surface (uncomfortable for the surgeon) or on a surgery table. Anesthesia is required for most procedures, and various local blocks and general anesthesia techniques available for use in bulls have been described.[1,2]

Some surgical procedures are performed to correct a condition that is or is suspected to be heritable.[3] It is the responsibility of the surgeon to be cognizant of the possibility of transmission of undesirable traits and to insist on bilateral castration if the condition is of a potentially serious nature.

SURGERY OF THE TESTES

Castration

Castration is the most common surgical procedure performed on bulls. Despite the relative simplicity of the procedure, many factors contribute to success or failure, including bull size, facilities, location, weather condi-

tance to mate, and on examination, prolapse of the preputial membrane and inflammation with swelling of the cranial one half of the penis and sheath can be seen. Treatment includes local and parenteral antibiotic therapy, along with hydrotherapy and bandaging. The prolapsed portion of the prepuce should be replaced and held in with a pursestring suture as soon as possible. Medical therapy should continue for 10 days to 3 weeks and until infection is controlled and healing is well under way. Surgery (circumcision) is indicated if the prolapsed prepuce cannot be replaced or if the healed laceration produces a stricture so that the bull's penis cannot be extended. Approximately 75% of bulls can be returned to service with some combination of therapy. It is difficult to justify surgical intervention in bulls worth less than $2000.

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tions, pre- and postsurgical environment, surgical method including speed and cleanliness, and other pre- and postsurgical stresses. The most common postsurgical complications include severe hemorrhage, wound infection with cellulitis and potentially fatal septicemia, inadvertent damage to the penis, and, rarely, intestinal herniation through the inguinal ring.

Two factors are important to consider in castrating bulls: (1) timing of the procedure and (2) selection of the method to be used. Bulls should be castrated early in life (at 1 to 3 months of age) because performing the procedure then is less stressful and more humane and better addresses animal welfare concerns. When given a growth-promoting implant, steers will reach market size about the same time as do intact bulls.[4] On ranches with a defined calving season, early castration is more easily coordinated. On those farms where calving occurs year round, however, castration at the ideal time necessitates performing castration in groups of animals several times a year. This approach is rarely used, and bulls tend to be castrated at weaning or sold as bulls and castrated at the feedlot or stocker operation. In the purebred industry, castration is purposely delayed to allow consideration of the better bulls for sale as breeding animals. Bulls can be castrated by open or closed techniques. Closed methods involve the use of rubber bands or the Burdizzo method (emasculatome). Open methods require surgical opening of the scrotum to remove the testicles. An economic evaluation of methods of castration has been reported.[4]

The objective of surgical castration is to remove both testes quickly in the cleanest environment possible. Depending on their size, bulls can be castrated while standing or recumbent and held by an assistant(s) or confined to a squeeze chute. Because of time constraints, the procedure usually is performed without anesthesia. Nevertheless, injection of 2% lidocaine directly into each testis will provide some analgesia. In the not-too-distant future, veterinary practitioners may well be compelled to provide analgesia for some procedures in cattle routinely performed without anesthesia for literally thousands of years.

To free the testes from the scrotum, a castrating knife* (Newberry knife) can be used. The knife is applied transversely across the base of the scrotum, closed, and pulled ventrally to open both scrotal sacs without removing any scrotal tissue, thereby preserving the scrotum (cod). An alternative method is to grasp the apex of the scrotum with one hand, stretch it ventrally, and with a scalpel in the other hand, excise the distal one third of the scrotal sac in one swift cutting motion. In both techniques, the tunica vaginalis should not be incised, and the testes are removed in a closed fashion. Once the testes are freed, the "one clean hand, one dirty hand" technique is used to free the spermatic cords from the surrounding fascia. The "dirty" hand grasps the testis (both testes, if small enough) and maintains ventral traction while the "clean" hand strips the fascia free by pushing the skin proximally. The testes are then removed by application of an emasculator proximal to each testis. Tension on the testis should be minimal at the time of emasculation. After the testes are removed, excess scrotal adipose tissue is trimmed from the wound and clostridial vaccines are administered if the patient was not immunized before the procedure.

An alternative to emasculation is ligation of each spermatic cord. Closed castration with a rubber band has been popular in smaller bulls for a number of years. A latex band (commonly used in sheep) can be applied proximal to both testes around the neck of the scrotum. It is important not to place the band so high on the scrotum as to risk injuring the penis. In recent years, castration of large bulls with rubber bands has become more popular with the development of newer equipment designed for the purpose. This method is particularly popular in feedlots. This closed technique uses an instrument* that allows rubber tubing to be stretched and clamped very tightly around the scrotal neck. Avascular necrosis of the scrotum and testes results. Done properly, this is a very effective castration method, and the animals are reported to undergo minimal stress.

All castrated animals should be immunized against clostridial organisms, particularly those causing blackleg and malignant edema. In addition, if rubber bands are used, protection against tetanus must be considered, particularly in large bulls in feedlot situations. Tetanus antitoxin can be used but is relatively expensive; vaccination with products containing tetanus toxoid at the time of castration have proven effective. After castration, animals should be placed in a clean environment, given the opportunity to exercise, and observed periodically over the subsequent 24 hours.

Epididectomy

Epididectomy is discussed later under "Preparation of Teaser Animals" later in the chapter.

SURGERY OF THE PENIS

The surgical procedures most commonly performed on the penis of bulls are for tumor removal, correction of penile deviations, closure of ruptures of the tunica albuginea, and separation of persistent penile frenulum.

Tumor Removal

The most common neoplasm found on the penis of bulls is a viral fibropapilloma. Such growths are common in young bulls housed together and undoubtedly are associated with the homosexual activity this management practice encourages. The lesion is caused by the bovine papillomavirus, which enters the skin through abrasions.

Affected young bulls frequently are presented with the complaint of hemorrhage from the preputial orifice, usually after coitus. Fibropapillomas also may be inci-

*Newberry Castrating Knife, Jorgensen Laboratories, Inc., Loveland, CO.

*EZE Bloodless Castrator, Wadsworth Mfg., Dublin, MT.

dental findings when a breeding soundness examination is performed. On rare occasions, the bull may be presented with the complaint of phimosis and swelling in the sheath. This can result when the papilloma is of sufficient size to cause pressure necrosis and swelling within the preputial cavity, which prevents penile extension.

Papillomas can be single or multiple and frequently are located on the free end of the penis and less commonly on the preputial skin. They tend not to be invasive, and although the tumor may appear large, the area of attachment often is relatively small. Blood supply to the fibropapilloma is significant, and removal of the tumor may require ligation or cautery.

The procedure can be performed with or without mild tranquilization, depending on lesion size, location, and the bull's demeanor. A pudendal nerve block may also be considered. Tail-stand restraint is sufficient to complete the surgery in most cases, however. To maintain extension of the penis, a cotton gauze bandage is used to get a firm grasp on the glans penis, and another is tied around the penis proximal to the tumor to act as a tourniquet and to assist in keeping the penis extended.

The penis is prepared for surgery with a surgical scrub. Local anesthetic is infiltrated to block the dorsal penile nerves by injection beneath the preputial skin on the dorsal surface of the penis at the level of the external preputial orifice.

The objective in removing a fibropapilloma is to excise it at the base of the lesion. To obtain adequate access to the base of an extensive tumor, a large portion of the mass can be simply cut away. At the base, an elliptical incision is made in the epithelium, leaving a stalk attached to the fibropapilloma. The stalk is ligated with size 0 polyglycolic acid* to prevent postsurgical hemorrhage. The stalk is severed, and the epithelium is closed over the wound. If the tumor is close to the urethra, it is advisable to insert a catheter as a guide to avoid accidental incision of the urethra.

In my experience, laser technology has proved to be a very effective method of removing penile warts, and as the costs of laser equipment decline in the future, it is hoped that this technology will become an economically viable alternative to standard surgical management.

Postoperative complications are rare after fibropapilloma removal. The bull should be withheld from breeding for 2 to 3 weeks, however, and examined before return to service. Regrowth of fibropapillomas occurs occasionally, and vaccination with a commercial or an autogenous wart vaccine may reduce the recurrence rate. Under certain management systems or in particular environments, penile fibropapillomas can affect a significant number of bulls. In these instances, management practices should be changed to avoid co-mingling large numbers of young bulls. A wart vaccine may be administered in an effort to reduce the incidence, although in my experience, vaccines have not been very effective.

*Dexon, Davis and Geck, American Cyanamid Co., Pearl River, NY.

Correction of Persistent Penile Frenulum

A persistent penile frenulum is recognized during a breeding soundness examination or when a young bull is placed in service for breeding. The condition arises when the penis fails to separate completely from the prepuce by 12 months of age. The penis and prepuce are fused at birth in the bull and separate as a result of testosterone stimulation beginning at 4 to 6 months of age. Persistent penile frenulum is suspected to be a heritable condition, and clients should be informed before corrective surgery is performed.

In most cases, a fibrous band extends from the prepuce and attaches to the ventral median raphe immediately caudal to the glans penis. On rare occasions, more than a single attachment point may be present, or the attached area is several centimeters long. The surgical procedure entails extension of the penis, surgical preparation of the area, and ligation of the frenulum with absorbable suture material at the preputial and penile extremities. The tissue is then severed between the ligatures. In those cases in which more than a single point of attachment is present, the other points are ligated and severed in a similar manner. Usually, hemorrhage is minimal. An antibiotic ointment can be applied postoperatively and the bull withheld from breeding for approximately 2 weeks. In cases characterized by a broad area of attachment between the penis and prepuce, corrective surgery must be discouraged.

Rupture Repair in Penile Hematoma

The classic penile hematoma is the result of rupture of the tunica albuginea and subsequent escape of blood from the corpus cavernosum penis (CCP) into and around the elastic layers surrounding the penis. During erection, very high blood pressures in the CCP have been measured.[5] Severe downward deviation of the erect penis during mating may cause a transverse tear through the tunica albuginea into the CCP and permit the release of blood. The injury in the tunica albuginea frequently is on the dorsal surface of the penis opposite the attachment of the retractor penis muscles; however, other rupture sites have been reported.[6,7]

The objective of the surgical procedure is to close the rent in the tunica albuginea, rather than allowing the injury to heal spontaneously. The view of some clinicians is that surgical closure of the rent results in a stronger union and a reduced likelihood of recurrence or shunt formation. This is a controversial point, however, and the decision to perform surgery should be based on elapsed time since the injury occurred, the economic value of the bull, and whether or not the owner places sufficient value on the bull to consider collecting and storing frozen semen after surgery. If semen preservation is not a course the owner wishes to consider, then surgery is not indicated. This recommendation is based on the fact that greater than 50% of bulls with this injury that are treated medically (with 10 to 14 days of systemic antibiotic therapy) successfully return to service.

The typical presenting signs include a swelling of variable size located immediately cranial to the scrotum. The

location alone often constitutes sufficient evidence to differentiate a penile hematoma from a peripreputial abscess. Preputial prolapse and swelling of the sheath frequently accompany penile hematomas and are the result of compromised venous drainage and development of dependent edema. The prolapsed prepuce may be traumatized, necessitating appropriate treatment. It is extremely rare for a peripreputial abscess to be located immediately cranial to the scrotum. These lesions typically are located in the mid-sheath area. In addition, the clotted blood in and around the elastic layers that surround the penis and adjacent tissue makes identification of the penis within the swelling difficult. If doubt still exists as to the contents of the swelling, aspiration (using a 16-gauge, 4-inch needle) after aseptic skin preparation and ultrasonography are alternative diagnostic procedures. If surgery is elected, it should be performed within 7 days of the injury, after which organization of the clot makes exteriorization of the penis, removal of the clot, and access to the tear in the tunica albuginea more difficult. Antibiotics should be administered as soon as the decision for surgery is made.

With the bull in right lateral recumbency and under general anesthesia or heavy tranquilization and local anesthesia of the incision site, the skin over the swelling cranial to the scrotum is shaved and prepared for surgery. The surgical area is draped, and an incision is made through the skin over the most prominent part of the swelling. This incision should be made in a cranioventral direction through the skin and subcutaneous tissue into the hematoma. Occasionally, the penis may be displaced and located just beneath the capsule of the hematoma, and care must be exercised in making the incision. This incision should be large enough to allow the surgeon's fingers into the cavity to remove the clotted blood. If little organization of the hematoma has occurred, the penis can be grasped and easily exteriorized. The area of the attachment of the retractor penis muscles is identified. Careful dissection through the elastic layer of the penis dorsal to this area usually reveals the rent in the tunic. Dissection of the elastic layer must be in a longitudinal direction to avoid damage to dorsal penile blood vessels and nerves. In fresh cases, dissection is relatively simple. In instances in which significant organization of the clot has already occurred, careful dissection and retraction are required to free the penis and facilitate exteriorization. Further careful dissection through the elastic layer is necessary to clearly expose the rent.

The edges of the rent are débrided and sutured with size 1 polyglycolic acid in a simple interrupted or cruciate pattern. It is not necessary to suture the elastic layer around the penis. The hematoma cavity should be thoroughly flushed with an antibacterial solution (5,000,000 IU potassium penicillin in 1 L sterile saline) before closure. The subcutaneous tissue can be sutured with a continuous pattern using size 0 polyglycolic acid. The skin is sutured with 0.6-mm nylon* in a continuous interlocking, cruciate, or horizontal mattress pattern. Postoperative care must include 10 days of systemic antibiotic therapy.

Complications are rare after aseptic surgery, but occasionally a seroma forms at the surgery site. This should not be drained, because abscess formation could follow. Within 7 to 10 days after surgery, the swelling will subside. The skin sutures can be removed at 12 to 14 days. The bull must have complete sexual rest for 60 days. After this period, the bull should be examined for the ability to successfully extend the penis and attain and maintain a normal erection. Sensitivity of the glans penis can be checked by application of a mild pain stimulus. If the bull's reproductive function appears normal, semen can be collected and frozen, or he can be returned to service at 60 to 90 days after surgery.

Four sequelae are possible after this procedure. The first is abscess formation, which requires drainage and usually results in peripenile adhesions and permanent inability to extend the penis. The second is desensitization of the glans penis due to damage to the dorsal penile nerves at the time of injury, during surgery, or after healing and return to service, when adhesions involving the nerves may break down, allowing the nerves to be severed during erection. Third, peripenile adhesions can develop, resulting in fixation of the penis; in the absence of postsurgical infection, however, this is rare. The fourth possible sequela is development of vascular connections (shunts) between the internal blood supply of the CCP and the external vasculature of the penis and prepuce. With formation of such shunts, the CCP is no longer a closed system, and sufficient pressure for normal erection cannot be maintained. The shunts can be identified by contrast radiography, and an attempt can be made to surgically excise them. Vascular shunts are quite rare, and the method for surgical correction has been described.[8]

Correction of Penile Deviation

Three forms of penile deviation are described in bulls. All three involve abnormalities of the apical ligament (AL) of the penis: The AL may be too short, resulting in an S-shaped deviation, which is rare; it may be too long, resulting in ventral deviation; or it cannot be maintained in its normal position when the penis is erect, resulting in a corkscrew or spiral deviation. Spiral deviation occasionally is observed in normal bulls during electroejaculation. Furthermore, the condition is known to occur in some bulls after intromission at the peak of erection. Only if the deviation is seen during natural mating attempts and intromission fails can an association between the deviation and subfertility be made.

The AL of the penis is an extension of the tunica albuginea that begins just proximal to the distal attachment of the prepuce. A band of collagen fibers extends forward on the dorsal midline of the penis and fans out laterally to encapsulate the distal portion of the penis where the ligament inserts back into the tunica albuginea. This ligament provides dorsal strength and support to the erect penis and maintains it in a straight profile, rather than allowing curvature downward and to the right, which is the natural tendency during erection. The ligament is not

*Braunamid, Jorgensen Laboratories, Loveland, CO.

fixed to the underlying tunica albuginea but is separated from it by a fascial layer. Thus, should the ligament be lengthened, a ventral deviation is possible. If it is too short or the bull has an unusually long penis, then an untreatable S-shaped deviation may occur. A spiral deviation occurs when the ligament slips off the dorsal surface to the left side and the distal penis becomes retracted into a spiral position.

Surgical attempts to correct either a spiral or a ventral penile deviation should be reserved for bulls of considerable economic value that must be used for natural service. If artificial insemination is an option, then surgical correction is not required. The objective of surgery is to provide dorsal strength and support in a longitudinal plane in the case of ventral deviation and to prevent lateral movement of the ligament in the case of spiral deviation. A single surgical procedure that utilizes an implant to provide support is used to correct both types of deviation. Other approaches to correct only spiral deviations have been reported, including the AL strip technique.[9]

The **implant technique** uses an autogenous or homologous strip of fascia lata from the cranial border of the biceps femoris muscle or carbon fiber material[10] to provide dorsal reinforcement of the apical ligament and fixation of the ligament to the tunica albuginea. Homologous strips of fascia lata can be harvested from freshly dead cattle and stored in 70% ethyl alcohol until used. All muscle, fat, and other loose connective tissue must be removed before preservation. Before use, the strip should be soaked in normal saline for 30 minutes. I much prefer this approach over harvesting autogenous fascia.

Two techniques are used to correct this condition in the bull, but only my preferred approach is described here. General anesthesia is induced and the penis fully extended. The penis and prepuce are prepared for aseptic surgery. A skin incision is made on the dorsal surface of the penis beginning just caudal to the glans penis and is extended proximally for 20 cm or to just before the point of attachment of the prepuce to the penis. In the proximal portion of the incision, a thin layer of elastic tissue is incised to expose the AL beneath. The thin subcutaneous fascial layer is then incised to expose the AL. The AL is incised along its dorsal midline to expose the tunicalbuginea. The edges of the AL are reflected to allow the implant material to be positioned beneath it. It is important to suture the implant as far proximally as possible and to keep it positioned on the dorsal midline. Simple interrupted size 0 polyglycolic acid sutures are placed in the proximal portion of the implant and into, but not through, the tunica albuginea. Sutures are placed similarly along the lateral borders of the implant for about one half the distance. The distal end of the implant is then sutured while under mild tension, and the remaining sutures are placed in the lateral borders. The objective is to "stretch" the implant into position when suturing. The implant should be trimmed when necessary to ensure a perfect fit. The AL is then closed over the implant with size 0 polyglycolic acid, with each simple interrupted suture passing through the implant. Proximally, the incised elastic layer should be sutured with size 3-0 polyglycolic acid in a closely spaced continuous pattern. The skin is closed with size 0 polyglycolic acid in a closely spaced interrupted pattern.

SURGERY OF THE PREPUCE

Repair of Preputial Injuries

Injuries of the prepuce frequently are encountered in bulls. Preputial injuries are more common in range bulls and may be the result of increased *Bos indicus* influence in the general cattle population. These injuries most often occur during the breeding season when the penis is extended. The outcome of the injury is greatly influenced by its location, depth of the laceration, interval between the insult and examination, and whether serious bacterial infection develops. Bulls with preputial injuries are presented with preputial prolapse with or without paraphimosis, swelling within the sheath, or phimosis without obvious swelling. Rarely are injuries of the prepuce discovered soon after they occur. Consequently, edema, infection, and fibrotic healing frequently are in progress when the patient is first examined. It is important to realize that except in cases of very minor injuries, the affected bull is unlikely to be capable of natural service for the remainder of the current breeding season. Even lacerations that heal without subsequent surgery need 45 to 60 days to heal sufficiently for the bull to return to service. Injuries that require surgery necessitate complete sexual rest for a period of at least 60 days after the surgery. This is in addition to 1 to 4 weeks that may be required for the prepuce to heal sufficiently for surgical intervention to be successful. Many bulls that appear initially to have grave injuries with a guarded prognosis can be successfully returned to service. The practitioner must weigh all of the costs required for treatment, including feed and maintenance, however, before embarking on an intensive and costly treatment protocol. Even in the face of a poor to fair prognosis, many clients will elect treatment when it is difficult to justify economically. Therefore, the practitioner must have a good understanding of the clinical management of preputial injuries to maximize the odds of success.[11]

Briefly, when bulls are presented with preputial injury and prolapse, the objective is to return the prepuce as quickly as possible to its normal position. Fresh lacerations are rarely seen, but when encountered they should not be sutured but rather managed medically and allowed to heal by second intention. At presentation, the prepuce frequently is affected by marked dependent edema. Reduction of the edematous swelling is best achieved by soaking the prepuce in a warm Epsom salt solution for 30 minutes, after which the tissue is dried and an antibacterial ointment is applied to the prolapsed tissue. A stockinette is then applied over the prolapsed portion of the prepuce. A soft plastic tube with smooth ends is inserted into the preputial cavity to facilitate drainage of urine. Beginning at the distal end of the prolapse, elastic tape is applied over the stockinette and continued proximally onto the hair of the sheath. Tape is then used to secure the tube within the preputial lumen. In bulls with pendulous sheaths, a sling of suitable

material is made and tied around the abdomen of the bull to attempt to keep the bandaged prepuce in a more horizontal position. Daily therapy (lavage with a nonirritating antibacterial solution) is administered through the tube. Initially, the bandage is changed every 48 hours. In a majority of cases, the prepuce can be returned to its normal position within 2 to 6 days. Once the prolapse has been reduced, the prepuce is maintained in position by wrapping the sheath with tape and inserting a tube into the preputial cavity. Lavage of the prepuce with antibacterial solutions can be continued by infusion through the plastic tube. A less preferred alternative is a purse-string suture placed in the preputial orifice to retain the prolapsed tissue. In those cases in which induration of the prepuce is so severe that the tissue cannot be returned to its normal position, amputation of the prolapsed portion is indicated.

When bulls are presented with distention of the sheath and phimosis, the swelling may be due to cellulitis or abscess. In those cases in which an abscess is not yet present, preputial lavage along with administration of systemic antibiotics often results in marked decrease in the swelling within a few days. No attempt should be made to extend the penis until healing is complete (which takes 4 to 6 weeks). Repeated attempts to extend the penis do not improve the likelihood of normal erection and protrusion after the lesion has healed but in fact may delay healing and increase scar formation.

When the laceration is deep into the elastic layers of the prepuce, with severe inflammation and abscess development, the prognosis is guarded. This is because even after the abscess is drained and healed, extensive fibrosis results in adhesions within the elastic layers and between the elastic layers and the subcutaneous tissue. The elastic layers are prevented from sliding over one another to allow penile extension. In my experience, attempts to surgically remove the scar tissue in such cases have largely been unsuccessful.

When an abscess does not extensively involve the elastic layers or the subcutaneous tissue and drainage through the preputial orifice occurs, systemic antibiotic therapy along with preputial lavage will eventually result in healing. Normal penile extension may be possible in rare instances, but usually surgical intervention is required to restore normal function.

Two surgical procedures have been described that can be used to remove excessive preputial tissue to prevent chronic prolapse, to remove chronically prolapsed preputial tissue that cannot be replaced within the preputial cavity, or to excise scar tissue in an attempt to restore normal preputial function: circumcision and definitive repair.

Circumcision

Circumcision is the surgical procedure utilized to electively remove excessive preputial tissue as a prophylactic measure or to remove badly injured and prolapsed preputial tissue that cannot be returned to its normal position. It is a procedure that, when performed properly for prophylactic purposes, is quite successful; however, when it is performed to remove seriously injured prolapsed tissue, postsurgical scarring and phimosis are common despite presurgical management as previously described. These cases often require subsequent surgery to remove scar tissue by resection and anastomosis (reefing). The circumcision procedure selected for a particular case depends on facilities available, cost, bull genotype, and the extent of the preputial lesion.

Amputation of the Prepuce

Amputation of the prepuce requires less stringent asepsis than that needed for resection and anastomosis and can be used as a prophylactic procedure to prevent preputial prolapse and to correct chronic preputial prolapse. The preferred technique is to place the bull in right lateral recumbency under moderate tranquilization or general anesthesia. The preputial hairs are clipped and the skin of the sheath shaved approximately 10 cm proximal to the orifice. The prepuce and sheath are prepared for surgery. The largest-diameter plastic tube that will fit is placed within the lumen of the prolapsed prepuce to the depth of the fornix. A 16-gauge, 4- to 6-inch needle is passed through the healthy preputial tissue proximal to the prolapse, to retain the tube in position. A tourniquet is tied around the prepuce proximal to the portion to be amputated. If necessary, 2% lidocaine is infiltrated around the prepuce just distal to the tourniquet.

Commencing distal to the tourniquet, a circumferential, full-thickness incision is made through the prepuce. This incision is made in an oblique plane so that the resultant orifice will be somewhat larger and reduce the risk of postoperative phimosis. The amputated portion of the prepuce is removed. Horizontal mattress sutures of size 2 polyglycolic acid are placed around the amputation line. These sutures should overlap one another around the entire circumference to control postoperative hemorrhage. It is imperative that the surgeon incorporate both layers of the prepuce in each suture. If the surgeon prefers, the amputation can be done in stages—that is, one third of the circumference can be incised and then sutured, then the next third, until the amputation is completed. The edges of the wound are then apposed with a simple continuous pattern of size 0 polyglycolic acid. The tourniquet and the plastic tube are then removed and a smaller tube is placed into the preputial cavity and secured with tape for 7 to 10 days as previously described.

Postoperative treatment with antibiotics is indicated for 5 days. Healing is complete in approximately 6 weeks, at which time the bull should be examined to determine ability to extend the penis. When this procedure is performed on relatively healthy tissue, the success rate is good; however, when it is performed on a badly injured, enlarged, and inflamed prepuce, the subsequent healing process often results in significant scarring, which will require removal by resection and anastomosis at a later date.

Resection and Anastomosis of the Prepuce

The combination of resection and anastomosis differs from preputial amputation in that the objective is to excise the fibrotic tissue that restricts normal extension of the penis. The fibrosis often is confined to a relatively

small area and usually is the result of healed lacerations. Resection and anastomosis may be required after preputial amputation when the procedure is performed under less than ideal conditions. This technique also is preferred in bulls with limited amounts of preputial tissue, in which amputation would severely restrict penile extension.

This procedure is performed when the healing process is complete or at least when the surgical field is healthy. Surgery must not be performed when obvious inflammation, edema, or exudate draining from the laceration is present.

The surgery must be performed under strict aseptic conditions. Careful dissection and hemostasis are essential to success. Ideally, general anesthesia is used after feed has been withheld for 48 hours and water for 24 hours. With the bull in right lateral recumbency, the skin of the sheath is shaved and prepared for surgery. The penis is manually extended as far as possible. Frequently, extension of the penis is impeded by the cicatrix. A pair of towel clamps is placed through the distal end of the penis, avoiding the glans penis and the urethra, and used to maintain traction on the penis. The portion of the penis and prepuce that is exposed is prepared for surgery. Complete extension of the penis requires that the restricting band of scar tissue be incised in a direction parallel to the penis. This is done with the scalpel while applying considerable traction on the penis. As the restriction eases, the penis and prepuce will eventually extend completely. This incision should be made with care to avoid incising deep into healthy elastic tissue. After the penis and prepuce are fully extended, a tourniquet is applied at the junction of the prepuce and the sheath. The previously unexposed portion of the prepuce is then prepared for surgery.

The extent of the scar tissue in the prepuce is now determined, and the locations for two circumferential incisions, one above and one below the cicatrix, are identified. Two interrupted sutures are placed in the preputial skin, one above and one below the intended incision sites, to assist in aligning the two preputial portions when the incision is closed. With the penis extended, the circumferential incisions are made through the preputial skin to expose the elastic layer. A longitudinal incision is then made that connects the two incisions that encircle the prepuce. The isolated section of prepuce is then dissected free and removed. The depth of dissection should be sufficient to remove as much scar tissue as possible. During dissection, several large blood vessels will be encountered. If they cannot be preserved intact, they must be ligated. When the dissection is complete and the scar tissue removed, the tourniquet is loosened. At this point, other significant bleeding must be controlled with ligation or electrocautery. Ligation of larger vessels is preferred, and it is essential that all bleeding be controlled before the two ends of the prepuce are sutured together. The surgical field is then lavaged with saline-antibiotic solution.

Closure can be accomplished with a single layer of horizontal mattress sutures using size 1 polyglycolic acid or catgut if the dissection was not deep into the elastic layers. Initially, three interrupted sutures are placed equidistant around the circumference of the prepuce. Frequently, the circumference of the proximal portion is greater than the circumference of the distal portion. To overcome this disparity, smaller bites are made in the distal wound edge compared with the proximal wound edge to compensate for the difference. The sutures must be placed close together in order to ensure that the wound is sealed.

To prevent urine irritation of the suture line, a clean plastic tube is inserted into the prepuce and bandaged in place to provide protection for the wound and allow passage of urine.

The bull must be confined for 7 days, after which the preputial bandage can be removed. Postoperative administration of antibiotics is indicated for 5 days. The bull must be isolated from all cycling females for 6 weeks, at which time the bull should be examined to determine ability to extend the penis and to evaluate the surgery site. If healing is complete and the penis can be extended normally, the bull can return to service after an additional 3 weeks of sexual rest.

The most common postoperative complication is wound dehiscence, which may be minor and without serious consequence or serious with return of scarring and phimosis. If the bull is of a *Bos indicus* breed, a second attempt at anastomosis and resection may be possible. It is unlikely that sufficient healthy preputial tissue will remain to allow the surgery to be performed a second time on bulls of the *Bos taurus* breeds.

Resection and anastomosis of the prepuce constitute an alternative to amputation of the prepuce. This procedure is readily performed on bulls as a prophylactic measure for preputial prolapse. In estimating the amount of preputial tissue to remove, a rule of thumb is that the remaining prepuce should be twice the length of the free end of the penis for the bull to attain normal penile extension.

Repair of Avulsion of the Prepuce

Avulsion of the prepuce usually is seen after use of an artificial vagina (AV) to collect semen from a bull. The injury involves partial separation of the prepuce from its attachment to the free portion of the penis. It usually occurs at the time of the ejaculatory lunge when the AV is excessively tight and restricts forward movement of the prepuce but not the free end of the penis, or when the caudal edge of the AV strikes the distal attachment of the prepuce.[12] This injury should be repaired immediately. The bull is restrained in a chute and tranquilized if necessary, and the penis extended. After the prepuce is cleansed, a local anesthetic is infiltrated in a ring block around the prepuce. If hemorrhage is significant, a tourniquet can be applied. The injury site is surgically prepared and examined. If significant separation of the elastic layers of the prepuce is seen, a closely spaced, simple continuous suture pattern using size 3-0 polyglycolic acid is completed first, and then the skin edges are brought into apposition with size 0 polyglycolic acid using a horizontal mattress pattern. If a tourniquet was applied, 15 minutes can be allowed for the blood to clot, after which the tourniquet should be removed.

Antibiotic ointment can be infused into the preputial cavity and gently massaged over the lesion daily for 5 days. The bull is withheld from service for 30 days.

PREPARATION OF TEASER ANIMALS

The successful use of artificial insemination in large beef and dairy herds can be enhanced by the use of estrus-detection (marker or teaser) animals. Steers, freemartins, or cows can be used after a period of androgen treatment (currently not legal in the United States). Intact bulls can be used after ensuring that their fertilizing capacity has been nullified through vasectomy or, more commonly, epididectomy. Surgical procedures can be performed to prevent intromission, which not only further safeguards against fertilization but decreases the possibility of transferring venereal diseases—in particular, trichomoniasis and campylobacteriosis.

The importance of preventing intromission in a group of cows or heifers depends on the management practices within that herd (e.g., consideration of bull size with heifers). In cases in which the female herd is closed and the teaser bulls are young, properly vaccinated (for immunization against infectious bovine rhinotracheitis/bovine viral diarrhea/parainfluenza type 3 [IBR/BVD/PI3], *Campylobacter,* trichomoniasis, leptospirosis, and *Haemophilus*), and free of brucellosis and tuberculosis, simple epididectomy is sufficient. Furthermore, teaser animals that are allowed intromission may perform over a longer period of time because of the absence of the "frustration factor" sometimes reported by cattlemen in bulls in which intromission is prevented. On the other hand, in herds with frequent introduction of animals with unknown or incomplete histories, intromission by the teaser bulls should be prevented. These bulls also should be vaccinated.

Nonsurgical Methods

Hormonal Induction

As stated earlier, no hormonal induction protocols are approved in the United States. Therefore, such protocols are not discussed further.

Epididectomy

Epididectomy is a relatively simple and safe procedure and can be performed more quickly than a vasectomy. The tail of the epididymis is located distally in the scrotum, and the objective of the procedure is to remove it. The surgery can be performed with the bull in either the standing or the recumbent position. The skin of the distal one third of the scrotum is shaved and prepared for surgery. Both testes are forced to the bottom of the scrotum and local anesthetic solution is infiltrated into the scrotal skin over the epididymal tail and into the epididymis. One testis is then released and the other is held in position ventrally in the scrotum. An incision is made over the epididymal tail, which must not be so long and deep as to accidentally allow complete prolapse of the testicle. The incision is made through the skin, tunica dartos, and the tunica vaginalis until the tail protrudes from the incision. The epididymal tail is then dissected free from its testicular attachments. With traction applied to the epididymis, two pairs of forceps are forced through the "loop" of the epididymal tail; one is then clamped across the vas deferens and the other across the most proximal part of the epididymal tail. Although not essential, it is preferable to ligate above both forceps with an absorbable suture material (size 1 polyglycolic acid). The tail of the epididymis is then removed by cutting below each forceps. An antibiotic preparation is applied to the site, and the incision may or may not be sutured. The procedure is repeated on the opposite testis. It is recommended that the teaser bull not be used for heat detection for approximately 3 weeks, to ensure that sperm remaining in the duct system have lost their capacity to fertilize.

Methods That Prevent Intromission

Several surgical procedures are described that in one way or another prevent the bull from gaining intromission.[13] Each procedure has its advantages and disadvantages in terms of cost, reliability, potential postsurgical complications, and technical ease. Intromission is prevented in one of three ways: (1) by translocation or deviation of the penis, (2) by adhering the penis or the prepuce in the retracted position, and (3) by prevention of erection.

Penile-Prepuce Translocation

Two techniques are described that translocate the entire penis and prepuce to the flank area of the bull: the tunnel and the Z-plasty techniques. Each procedure is best performed well in advance of the breeding season and should be limited to use in bulls that weigh 500 to 600 pounds. The surgery is performed with the bull in right lateral or dorsal recumbency under heavy tranquilization or general anesthesia. The skin of the caudal ventral abdomen from the left flank to the right side of the midline is shaved and prepared for surgery. My preferred technique is the tunnel technique, so only this method is described.

Two percent lidocaine is infiltrated around the preputial orifice and caudally on the ventral midline of the sheath to a point just cranial to the scrotum. Lidocaine also is injected into a circular area immediately above the fold of the left flank to which the prepuce will be translocated. It is important not to get this translocation site too high in order to avoid postoperative urine scalds on the skin nor too low, as the bull may learn to gain intromission.

The first incision is made completely around the preputial orifice approximately 3 to 4 cm from the cutaneous junction. The incision is then extended caudally for about 25 cm along the midline of the sheath. The incision is deepened, and by dissection the prepuce is freed completely from the abdominal wall. Care should be exercised to preserve the caudal blood supply to the prepuce. Hemorrhage may be extensive, particularly when the preputial orifice is dissected, and all bleeding vessels must be ligated. It also is critical not to incise into the preputial cavity. To avoid this, a small-diameter tube can be inserted into the prepuce to act as a guide.

After the prepuce is freed, a circular incision approximately 6 to 7 cm in diameter is made through the skin, and the cutaneus truncus muscle above the fold of the flank and the abdominal tunic is exposed. A long pair of forceps (e.g., Knowles cervical forceps) is then passed subcutaneously from the flank incision to the caudal end of the ventral midline incision to emerge cranial to the scrotum. The forceps are partially opened, and the fascia between the jaws is incised to allow the forceps to pass through. A subcutaneous tunnel is created through which the preputial orifice and the prepuce will be pulled. To avoid contaminating the tunnel with debris, a sterile obstetric sleeve is placed over the freed prepuce before it is grasped with the forceps and pulled through the tunnel. It is important to ensure that the prepuce is not twisted while being translocated.

Closure consists of suturing the cutaneus truncus muscle to the subcutis of the relocated preputial orifice with size 0 polyglycolic acid using a simple interrupted pattern. The skin surrounding the preputial orifice is apposed to the skin of the flank with nonabsorbable suture (0.6-mm nylon) in a cruciate or horizontal mattress pattern. The midline incision is closed with nylon using a continuous pattern and periodically suturing deeper to include the abdominal wall fascia, in an attempt to obliterate space where fluid might accumulate. The original circular preputial incision may be left open for drainage provided that the animal is returned to a clean area.

To render bulls infertile and reduce the liability of the surgeon for unwanted matings, a bilateral epididectomy should be performed after completion of the penile translocation procedure. Postoperative administration of antibiotics is indicated, as well as daily application of petroleum jelly around the preputial orifice for 3 to 4 days. Complications include dehiscence, abscess formation, and severe urine scalds.

Penile or Prepuce Fixation

Several fixation techniques have been described that are reportedly successful for prevention of intromission[13]; however, with these fixation techniques, it seems reasonable to expect that bulls may experience some degree of pain when mating is attempted. Information is lacking regarding the comparison of longevity of effectiveness of the various teaser surgery techniques that involve some alteration of normal penile function. On the basis of limited experience with this technique, I believe that teaser bulls with penile or preputial fixation tend not to be as functional over time as are bulls with penile deviations.

The fixation technique described here involves suturing the tunica albuginea of the penis to the abdominal tunic. Fibrosis and adhesions result, preventing penile extension. With the bull tranquilized and in right lateral recumbency, a clean plastic tube is passed into the preputial orifice and used to identify the caudal limit of the cavity. The skin of the abdomen between the scrotum and the preputial orifice is shaved and prepared for surgery. Lidocaine is infiltrated along a line parallel to the penis. An 8- to 10-cm skin incision is made over the penis beginning just caudal to the posterior limit of the preputial cavity. The incision is deepened through the subcutaneous tissue and the elastic layer to expose the tunica albuginea. The dorsal lateral surface of the penis is débrided, as is the adjacent abdominal tunic, and three or four nonabsorbable sutures are placed through the dorsolateral aspect of the tunica albuginea and the abdominal tunic. The subcutaneous tissue is closed in a continuous pattern with size 0 polyglycolic acid and the skin is apposed with 0.6-mm nylon. A bilateral epididectomy is performed to ensure that the bull is not capable of a fertile mating. Postoperative edema is expected, but recovery usually is uneventful. A period of 30 days is allowed for healing before the bull is returned to service.

A similar technique has been used successfully to adhere the prepuce to the abdominal tunic. In this procedure, the bull is tranquilized and cast in right lateral recumbency. The skin over the sheath is prepared for surgery and lidocaine is infiltrated along the left dorsolateral aspect of the sheath beginning 6 cm from the preputial orifice and extended posteriorly for 20 cm. A 15-cm skin incision is made along this line and with blunt dissection the prepuce is freed to allow it to be pulled from the incision. A significant portion of the elastic layer of the prepuce is excised without penetrating into the cavity. After excision of a portion of the elastic layer, three or four sutures are placed through the prepuce and into the abdominal fascia using 0.6-mm nylon. A continuous suture can be placed in the subcutaneous tissue using absorbable material, and the skin is closed with nylon. The fibrous tissue formed as the prepuce heals in the absence of an intact elastic layer in a portion of the prepuce results in adhesions between the prepuce and the body wall, so that penile extension is not possible. Again, as an added precaution, an epididectomy should be performed. In approximately 30 days the bull will be ready for service.

Other Techniques

Other procedures have been used in the past to produce teaser bulls but are not discussed here in detail because they have, for various reasons, not become as popular as those previously described. These include the corpus cavernosal block, penectomy, and preputial ring techniques.[13]

SURGERY OF URINARY STRUCTURES

Calculi occasionally lodge in the urethra of both steers and bulls. Steers are diagnosed with the condition more frequently than are bulls because of the smaller diameter of their urethras. Several factors play a role in the development of urinary calculi, including diet, water intake, and urine pH. The calculi found in feedlot steers are mostly phosphates and are multiple, soft, and smooth, whereas those recovered from steers at pasture are oxalates or silicates and are rough-surfaced and hard. Urethral blockage can occur at any level of the urethra, but the most common site for initial blockage is the distal curve of the sigmoid flexure.

When the diagnosis is made early, the calculus or calculi can be removed from the urethra through a low

urethrostomy. More often, however, the diagnosis is made after significant periurethral urine leakage with inflammation and possibly rupture of the urinary bladder. Subcutaneous edema and cellulitis often are encountered at this stage, and the objective of surgery is to restore urine flow as quickly as possible to salvage the steer for slaughter. Because this is the most common scenario, only a technique for high urethrostomy is described.

The preferred site for urethrostomy in these animals is the ischial area. The procedure can be performed on the animal standing with epidural anesthesia. An area of skin is shaved and prepared for surgery beginning at the level of the ischium and continued ventrally for 30 cm. The skin and subcutaneous tissue are incised on the midline beginning at the level of the tuber ischii, and the incision is continued ventrally for 15 to 20 cm. The penis is located deep in this area, and considerable dissection is required to free the penis and allow it to be grasped. The penis is then pulled out through the incision and transected so that a 4- to 5-cm stump will protrude from the ventral commissure of the skin incision. A frequent mistake in transection of the penis is to leave a stump of insufficient length. In transection of the penis, the dorsal penile vessels should be isolated and ligated before the penis is severed. The proximal portion of the penis is then anchored to the skin with nonabsorbable horizontal mattress sutures placed through the skin, tunica albuginea, and corpus cavernosum penis on both sides of the stump. It is important that the stump is not sutured to the skin in such a manner as to create an acute bend, which would restrict the flow of urine. The urethra can be incised longitudinally and its edges sutured to the skin with 2-0 polyglycolic acid in a simple continuous pattern. The skin above the penile stump is apposed with 0.6-mm nylon.

Postoperatively, animals should be observed for normal urination. Treatment with antibiotics is indicated for animals not destined for immediate slaughter. Ventral accumulation of urine can be relieved with several deep skin incisions made parallel to the prepuce.

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CHAPTER 34

Techniques for Artificial Insemination of Cattle with Frozen-Thawed Semen

RAYMOND L. NEBEL

In the modern commercial dairy herd, management of the artificial breeding program is a major responsibility. It requires constant attention and expertise by personnel responsible for semen handling and insemination. Whether the responsibility is contracted to a professional artificial insemination (AI) technician or is assigned to employees of the farm, the requirements for success are the same. The artificial breeding program depends on the inherent fertility, health, nutrition, and management of both the cows and the bulls, as well as on the ability of the handlers to preserve, store, and deliver an adequate dose of viable semen to the cow at the proper time during the estrous cycle.

The primary objective in handling semen properly is to conserve the fertile life of sperm until deposition in the female. This is accomplished by minimizing exposure of semen to injurious conditions, which have additive effects that lower the viability and fertile life of sperm. With frozen semen, the storage temperature of liquid nitrogen stops the life processes, allowing semen to be stored indefinitely if it is maintained at very low temperatures. The critical temperature appears to be –80°C. A point of paramount importance is that although it is easy to maintain frozen semen at a safe temperature, it also is easy to destroy its quality in a few moments of carelessness.

High conception rates require proper insemination techniques. The highest-quality semen placed in the healthiest cow at the proper time before ovulation will not produce a calf if the breeding techniques are not up to par. The mechanics of passing the insemination pipette through the cervix necessitate practice and should be periodically reviewed with the aid of professionals. Sanitary methods are critical to good insemination technique. Good technique also means that each insemination is performed within a reasonable amount of time. Consistently following a step-by-step procedure will assist in achieving good conception rates.

SEMEN TANK MANAGEMENT

The semen storage tank is actually a large metal vacuum-sealed liquid nitrogen refrigerator encased within an extremely efficient insulation system. With proper attention and handling, most liquid nitrogen semen storage tanks give years of trouble-free service, but all storage tanks eventually fail. The semen storage tank consists of two separate chambers. Layers of aluminum foil and specially designed paper fill the space between the two chambers. Air is removed and a partial vacuum is created in the area between the two chambers. This vacuum aids in the insulation and is the major effective property of the storage tank. A specially designed stopper plugs the tank's neck tube, insulating liquid nitrogen and semen from outside air. The tank is not airtight, however. Because liquid nitrogen cools by slowly boiling and releasing gases, a tightly stoppered tank might explode. The specially designed plug allows nitrogen gas to safely escape from the inner chamber. Storage tanks keep semen indefinitely at –196°C as long as any liquid nitrogen is present, but a depth of at least 5 cm of liquid nitrogen should always be maintained as good insurance against possible service delays. The normal field holding time or time allowed between liquid nitrogen refills varies according to the tank model and the number of times it is opened. The liquid nitrogen level should be monitored weekly with a measuring stick.

Technical advances in design and construction have produced storage tanks with liquid nitrogen holding times of 6 to 9 months. Although semen tanks are well constructed, they are still susceptible to damage from mishandling. Damage to the liquid nitrogen tank is most likely to occur when the tank is moved, so excessive movement of the tank should be avoided. The inner chamber of the tank, which contains the liquid nitrogen, is actually suspended from the outer shell by the neck tube. Any abnormal stress on the neck tube, which can be caused by sudden jarring or any excessive swinging motion, can crack the neck tube, resulting in vacuum loss and tank failure. Failure to secure the tank in a moving vehicle can result in not only increased probability of damage to equipment but also increased risk of injury to the driver or passengers if liquid nitrogen is spilled inside the passenger compartment of the vehicle.

To ensure maximum holding time, the tank should be kept in a cool and dry location away from direct sunlight, in a clean and well-ventilated area. The tank should be located in an area where it can be observed frequently to detect excessive nitrogen evaporation and development of frost around the outside neck of the storage tank. Drafts from milk coolers and furnaces should be avoided, but ventilation should be sufficient to prevent possible

user suffocation, which can be caused by too high a proportion of nitrogen gas in the air. The tank should be protected from corrosion by being elevated above concrete or wet floors. A wooden dolly equipped with wheels so that it can be moved easily is ideal for tank storage. The tank should be located where it will be safe from children and vandals or locked to secure and protect semen. Particular attention must be paid to the neck and vacuum fitting. Accumulation of frost on these fittings indicates that the vacuum insulation has been lost and liquid nitrogen has been evaporating rapidly. If frost build-up is observed, a functional tank must be located and the semen transferred to it immediately. The viability of semen in the tank should be evaluated before extensive use.

As with any well-managed business, an accurate inventory of location and quantity of the semen stored in the liquid nitrogen tank is important. This is necessary not only for accounting and audit purposes but, more important, to avoid any unwanted exposure of semen during searching for the desired sire's semen before removal for thawing. A simple semen tank inventory log enables the exact location and quantity of semen remaining to be determined quickly.

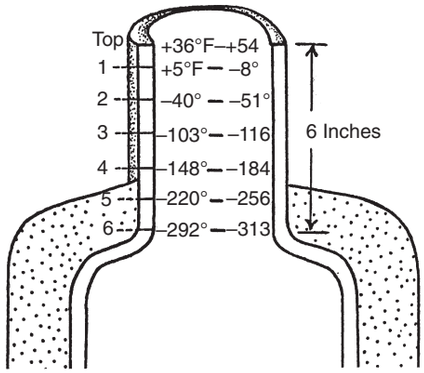

Fig. 34-1 Temperature range found in the neck of the semen storage nitrogen tank. (From Saacke RG: Concepts in semen packaging and use. Proceedings of the Eighth Conference on Artificial Insemination in Beef Cattle of the National Association of Animal Breeders, 1974, p 15.)

Storage of Frozen Semen

Maintenance of low temperatures is important to the successful storage of frozen semen. In 1957, Van Demark and coworkers[1] compared the motility of semen frozen to and stored at –79°C, –65°C, –51°C, –37°C, and –23°C. After 1 day of storage, the motility of semen stored at –37°C and –23°C was basically zero. Semen stored for 1 day at 79°C had 38% motility, compared with 29% and 14% motility for 65°C and 51°C storage, respectively. In studies in which –79°C storage was compared with –96°C[2] or –92°C and –196°C 79°C was inferior to the lower temperatures when judged by maintenance of sperm motility as measured on thawing.

The reasons that subzero temperatures of –79°C and above cannot adequately preserve sperm are unclear and probably are quite complex. Rapatz,[2] however, has proposed that rearrangement in the crystalline structure of frozen semen (recrystallization) may be one cause. This phenomenon has been observed to occur in frozen semen down to a temperature of –80°C. Below –80°C the structure of ice is more stable, and below –100°C it is very stable. Although no direct evidence exists for recrystallization, a correlation has been found between storage injury of spermatozoa and recrystallization.

In addition to the obvious error of permitting a liquid nitrogen storage tank to go dry, stored semen also may be exposed to adverse high temperatures when straws are being removed for thawing. Figure 34-1 shows the temperature gradient that exists in the neck of a typical liquid nitrogen storage tank. As may be noted, dangerously high temperatures prevail in the upper third of the neck of the tank where canes and goblets are raised for removal. When straws are exposed to these temperatures the semen temperature rises quickly. The thermal response of semen in 0.5-ml straws exposed to temperatures of –22°C (2 inches from the top of the tank) and 5°C (1 inch from the top of the tank) is shown in Figure 34-2. The time required to reach –100°C to –80°C, which is the beginning of ice recrystallization, is approximately 10 to 12 seconds for both temperatures. Thermal injury to sperm is permanent and cannot be corrected by returning semen to the liquid nitrogen. For optimal maintenance of sperm viability, canisters and canes containing semen should be raised into the neck of the tank only for

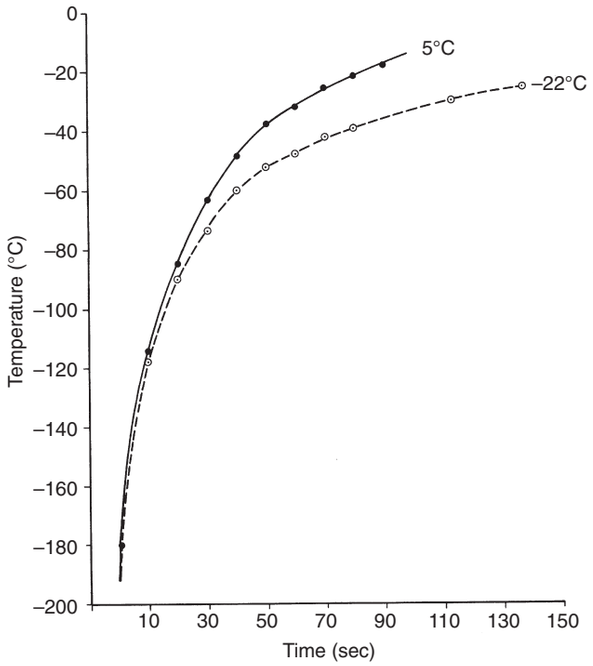

Fig. 34-2 Thermal response of semen in 0.5 mL French straws when exposed to 5°C and –22°C (temperature found in the *upper portion* of the nitrogen storage tank). (From Saacke RG, Lineweaver JA, Aalseth EP: Procedures for handling frozen semen. Proceedings of the Twelfth Conference on Artificial Insemination in Beef Cattle of the National Association of Animal Breeders, 1977, p 49.)

the time required to retrieve a single straw. This time should not exceed 5 to 8 seconds.

SEMEN-THAWING PROCEDURES

The recommendation for thawing of semen frozen in straws is not the same for all AI organizations. For optimal results, the recommendations of the semen processor should be followed. All U.S. semen processors recommend warm-water thawing of straws for periods ranging from 10 to 60 seconds. The situation on most farms is that dairy producers purchase semen from many AI organizations, but only one thawing procedure is performed. The National Association of Animal Breeders (NAAB) has recommended that when specific thawing requirements are in doubt, the straw should be immersed in 30° to 35°C water for a minimum of 40 seconds. Most U.S. AI organizations are members of the NAAB. Based on the scientific literature, warm-water thawing of semen at 35° to 37°C seems most appropriate and safest for vapor-frozen semen packaged in 0.5-ml straws.[3–8]

It has been reported that using a nonthermostatically controlled water bath allows batch thawing of straws (up to 20 at a time) without compromising semen quality when the ambient temperature is approximately 20°C.[9] As many as 10 straws can be thawed simultaneously in a thermostatically controlled thawing bath without significantly decreasing sperm viability. Of note, sperm that remain in a nonthermostatically controlled bath tend to cool over time; this finding may explain the slightly better sperm viability in comparison with that of semen thawed with a thermostatically controlled bath, because the metabolism of cells is lower in the nonthermostatically controlled bath. Thermostatically controlled water baths are designed to maintain a temperature of approximately 35°C.

Regardless of the number of straws thawed simultaneously, straws should be agitated immediately after being plunged into the water, to prevent them from freezing together during the thaw. Duration of incubation time in the bath should be minimized but also depends on the ambient temperature. Ambient temperatures below 20°C dictate that straws remain in the bath until immediate insemination is possible. By contrast, high ambient temperatures allow the immediate removal of straws once thawed; thus, holding time occurs in the insemination rod.

SEMEN HANDLING AFTER THAWING

Regardless of what type of water bath is used, all water should be thoroughly removed from the straw before it is cut. Osmotically, exposure of the 0.5-ml dose of semen to as little as one drop of water results in irreversible cell injury. Rarely, a straw may be defective and leak, permitting water to enter during thawing. If this is suspected, that straw should not be used.

A major concern with warm-water thawing is the danger of cold shock caused by mishandling of the semen after thawing. **Cold shock** is the irreversible injury to sperm caused by a rapid decrease in semen temperature above freezing after thawing. Cold shock occurs when semen is thawed and then subjected to cold environmental temperatures before insemination. The severity of damage depends on rate and span of temperature drop. It results in loss of motility, metabolic activity, and fertilizing potential and is believed to involve irreversible changes in the outer plasma membrane.

Cold shock occurs most frequently when breeding is undertaken in cold weather and particularly when warm-water thawing is used. As might be expected, the high surface-to-volume ratio of the straw makes it vulnerable to cold shock. Saacke and coworkers,[10] using the 0.5-ml French straw, measured the effect of static ambient temperatures—21°C, 4°C, and 16°C—on rate of temperature drop in semen after thawing during preparation of the inseminating rod (Figs. 34-3 to 34-5). Preparation of the inseminating rod, from thawing of the straw at 35°C to the protection of the loaded rod by tucking it against the body, required approximately 1 minute. At each ambient temperature, the rod was prepared at that temperature or warmed by being rubbed quickly with a paper towel several times over the length of the rod. As is illustrated in Figure 34-3, the temperature drop was only 3° to 6°C for a 21°C ambient temperature, and warming the rod was effective in minimizing the temperature drop. At 4°C and –16°C (see Figs. 34-4 and 34-5), the drop in semen temperature was approximately 15°C and 20°C, respectively. Because severity of sperm injury due to cold shock is related to the rate of cooling, warming the rod would be of little value for the two coolest ambient temperatures because it would only

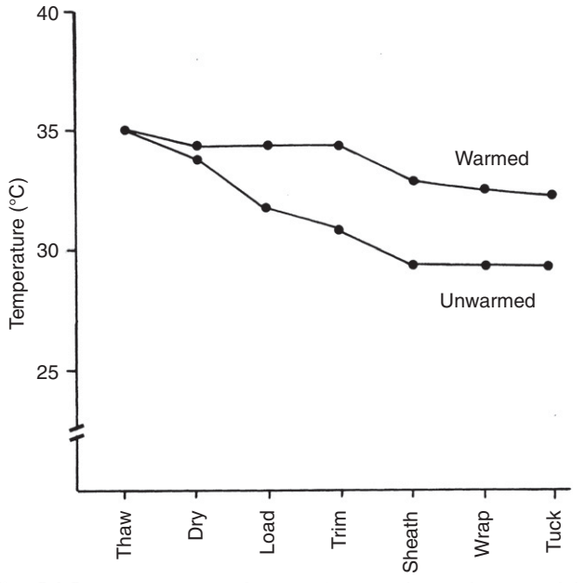

Fig. 34-3 Temperature of semen in 0.5-ml French straws following a 35°C warm-water thaw during preparation of the artificial insemination rod at a 21°C ambient temperature. Preparation of the rod from thaw to tuck required approximately 1 minute. The rod was warmed by being rubbed rapidly with the hand *(warmed)* or was kept at ambient temperature *(unwarmed)*. (From Saacke RG, Lineweaver JA, Aalseth EP: Procedures for handling frozen semen. Proceedings of the Twelfth Conference on Artificial Insemination in Beef Cattle of the National Association of Animal Breeders, 1977, p 55.)

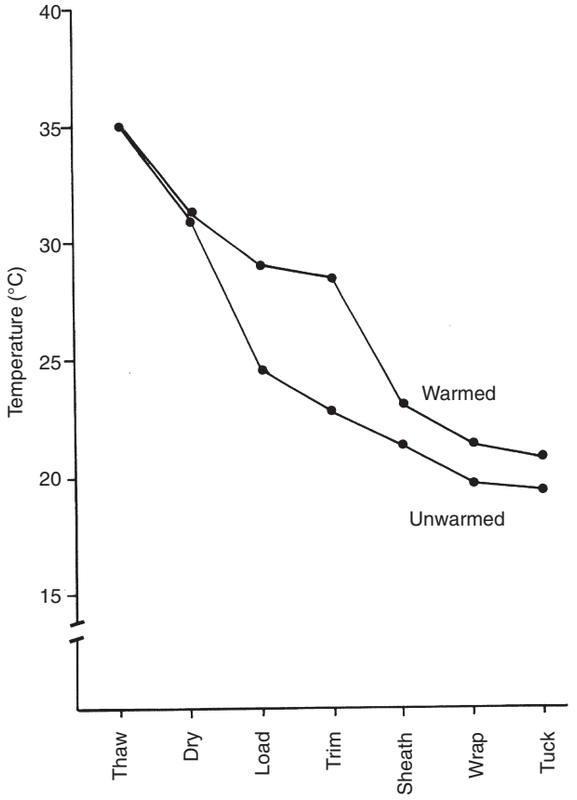

Fig. 34-4 Temperature of semen in 0.5-ml French straws after a 35°C warm-water thaw during preparation of the artificial insemination rod at a 4°C ambient temperature. Preparation of the rod from thaw to tuck required approximately 1 minute. The rod was warmed by being rubbed rapidly with the hand *(warmed)* or was kept at ambient temperature *(unwarmed).* (From Saacke RG, Lineweaver JA, Aalseth EP: Procedures for handling frozen semen. Proceedings of the Twelfth Conference on Artificial Insemination in Beef Cattle of the National Association of Animal Breeders, 1977, p 55.)

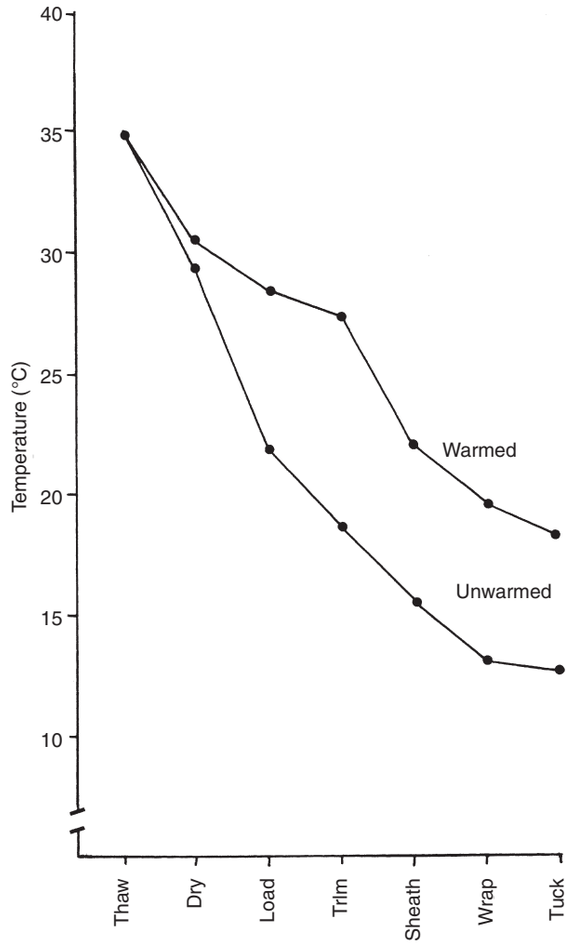

Fig. 34-5 Temperature of semen in 0.5-ml French straws after a 35°C warm-water thaw during preparation of the artificial insemination rod at a 16°C ambient temperature. Preparation of the rod from thaw to tuck required approximately 1 minute. The rod was warmed by being rubbed rapidly with the hand *(warmed)* or was kept at ambient temperature *(unwarmed).* (From Saacke RG, Lineweaver JA, Aalseth EP: Procedures for handling frozen semen. Proceedings of the Twelfth Conference on Artificial Insemination in Beef Cattle of the National Association of Animal Breeders, 1977, p 55.)

postpone the temperature drop. It is clear that in cool weather, precautions against cold shock must be implemented during preparation of the inseminating equipment.

Because of potential cold shock, many investigators have questioned the wisdom of the warm-water thaw, particularly when breeding will take place during cold weather. Fleming and co-workers,[11] using the 0.5-ml French straw, studied the effect of different thaw rates on motility and acrosomal integrity of sperm after 3 hours of incubation after thaw. In their comparison, they included a 35°C thaw followed by immersion of the straws in a 5°C water bath. This treatment achieved a maximum cold shock. Their results (Table 34-1) show that both motility and acrosomal integrity are adversely affected by cold shock, when compared with a 35°C thaw without cold shock; however, air thaw and 5°C water thaw were still inferior to the 35°C thaw followed by cold shock.

In cold weather, either of the following precautions seems warranted: (1) provide a sheltered heated area for breeding, or (2) provide a sheltered heated area for semen thawing and loading of the insemination pipette near the animals to be bred; then insulate the inseminating equipment and carry it to the breeding chute. Freezing weather brings the possibility of refreezing thawed semen. This can be classified as a disaster with regard to semen quality. Regardless of the thaw procedure recommended, conditions causing a sudden drop in the temperature of the semen should be avoided.

SEMEN THAWING AND HANDLING TIPS

It is essential that frozen semen be handled and thawed carefully and properly to maintain sperm viability for

Table **34-1**

Effect of Thaw Bath Temperatures and Cold Shock on Recovery by Spermatozoa in 0.5-ml French Straws

	AFTER INCUBATION FOR 3 HR AT 37° C	
Thaw Method	**% Motile**	**% Intact Acrosomes**
5° C	30.3^{AB}	31.2^{A}
Air	21.4^{C}	26.4^{A}
35° C	51.4^{B}	61.0^{B}
35° C, then 5° C cold shock	41.1^{ABC}	44.6^{C}

^{ABC}Different superscripts designate significant differences ($P < 0.05$) in each column.
From Fleming WN, Olar TT, Mitchel JR: Techniques for evaluation of frozen bovine semen at Curtiss Breeding Service. Proceedings of the Sixth Technical Conference on Artificial Insemination and Reproduction of the National Association of Animal Breeders, 1976, p 90.

optimal results. Insemination equipment should always be kept clean, dry, and warm. A thermometer should be used to obtain the proper water temperature. The thermometer should be checked for accuracy at least every 6 months with a reference mercury thermometer.

When removing the straw from the nitrogen tank, the handler should gently shake the straw to remove any liquid nitrogen that may be retained in the cotton plug end of the straw. The thaw should be timed with a watch to avoid guessing. While the semen is thawing, the handler warms the insemination rod by rubbing it briskly with a paper towel. Once it has been warmed, the handler places the insemination rod within his or her clothing so that it will be close to the body to maintain warmth. After the semen is thawed, the straw is dried thoroughly with a paper towel and protected from rapid cooling. The air space in the straw should be adjusted to ensure that no semen will be lost when the end of the straw is cut off. This can be done by slightly flicking the wrist while holding the straw at the crimp-sealed end. Only sharp scissors or a specially designed straw cutter should be used to cut the straw. The straw is cut "square" at a 90-degree angle to achieve a good seal with the sheath. The assembled insemination rod is wrapped in a clean, dry paper towel and tucked within clothing for transport to the cow. Finally, the cow should be inseminated within minutes after the semen has been thawed. The period of time between removing the straw from the tank and depositing the semen in the cow should not exceed 15 minutes.

SEMEN DEPOSITION

To reiterate, high conception rates require proper insemination techniques. The highest-quality semen placed in the healthiest cow at just the right time will not produce a calf if the breeding technique is not performed properly. The mechanics of passing the inseminating rod through the cervix are not covered here; for further information, AI organizations or other institutions that provide training in this technique should be consulted. Practice is required to develop the skill, which should be learned and periodically reviewed with the assistance of professionals.

One of the most critical parts of the insemination technique is depositing the semen anterior to the cervix. A majority of sperm deposited by natural service is lost from the reproductive tract shortly after being deposited. The major reason why sperm numbers can be markedly lower in each dose of semen used in AI is that the cervix, which is the major barrier to sperm transport, is bypassed in correct semen deposition with AI. Senger and co-workers[12] reported a significant increase in fertility when bilateral cornual insemination was performed. Seguin,[13] Zavos and co-workers,[14] and Williams and co-workers[15] all reported improved fertility when cornual insemination was performed. Marshall and co-workers[16] reported a slight but not statistically significant negative effect on conception when bicornual insemination was performed (54% versus 50%, respectively). A tendency for improvement was observed, however, when inseminators who were achieving low conception rates by attempting to deposit semen in the uterine body were retrained to deposit semen in both uterine horns. Overall, agreement on the effect of bicornual insemination is still lacking, because neither a consistent benefit nor significant negative effect has been demonstrated at this time.

Gallagher and Senger,[17] using a vaginal sampling technique, showed that sperm retention by the female reproductive tract did not differ when cornual insemination was compared with uterine body insemination; sperm movement into the vagina from the uterus was about the same in both cases. When sperm were deposited in mid-cervix, retrograde sperm loss was almost twice the loss observed after cornual deposition. If cornual insemination has any advantage in terms of fertility, it probably is due to the elimination of errors associated with attempting to deposit the semen in the uterine body but, instead, depositing a portion or all of the inseminate in the cervix. Anterior cornual insemination does not appear to result in greater retention of sperm than that achieved with insemination into the uterine body. Although the factors that control sperm transport and retention are still poorly understood, it is clear that retrograde transport of sperm occurs regardless of the site of deposition. Also, deposition of sperm in the anatomic vicinity of fertilization does not enhance fertility.

SUMMARY

Many livestock producers are breeding their own cows, and others are contemplating such a change in management. The level of success ranges from excellent to frustratingly low. Like many technologies of today, AI requires both skill and management ability. Through conscientious effort and attention to detail, the herd manager–inseminator can be successful. A thorough knowledge of the risks and pitfalls avoids problems and helps to ensure satisfactory results.

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CHAPTER 35

Clinical Reproductive Physiology of the Cow

JEFFREY S. STEVENSON

Reproduction drives the production cycle in both beef and dairy enterprises and is of major economic consequence. Maximizing the reproductive potential in cattle requires the understanding and application of many principles basic to the various disciplines of animal and veterinary science, including genetics, nutrition, physiology, and theriogenology, as well as management intervention.

This chapter reviews the most current information on the major endocrine and physiologic events that are known to be associated with the onset of sexual maturation or puberty; the physiology of the estrous cycle, pregnancy, parturition, and periparturient period; and the postpartum transition from anestrus to the onset of estrous cycles. Because of the extent of literature covering these topics, the reader is referred to several key articles and major reviews for further in-depth information.

ONSET OF SEXUAL MATURATION

Increasing reproductive efficiency in beef and dairy herds depends on the timely introduction of replacement heifers into the breeding herd. Heifers should have their first calf by 2 years of age. Maximizing the proportion of

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ONSET OF SEXUAL MATURATION

Increasing reproductive efficiency in beef and dairy herds depends on the timely introduction of replacement heifers into the breeding herd. Heifers should have their first calf by 2 years of age. Maximizing the proportion of

beef heifers that calve early in the calving season leads to heavier calf weaning weights, allows for timely rebreeding, and increases their herd longevity. To maximize lifetime milk production in a dairy operation, replacements also must calve as 2-year-olds in order to recoup rearing costs. Achieving this goal in either type of production unit requires appropriate heifer development and timely onset of puberty and first conception.

Puberty generally is defined as the onset of the first estrus associated with a potentially fertile ovulation that is followed by a luteal phase of normal duration. Prepubertal heifers may have at least one anovulatory estrus that precedes their first normal cycle (13% to 22% of heifers at an average of 3 months before puberty), often called a nonpubertal estrus. Puberty represents only the onset of sexual maturation, which actually is not achieved until the female reaches the maturity characteristic of her breed. This is recognized more easily in a litter-bearing species as the time when maximum ovulation rate or litter size is achieved.

Factors Influencing Onset of Puberty

The onset of puberty, as well as of nonpubertal estrus, is influenced by several factors, including age, genotype, season, body weight, nutrition, and social rearing environment.[1]

Genotype

The breed composition of heifers alters age at puberty, because size at puberty is determined genetically. Numerous studies have demonstrated differences in age and weight at puberty among various breeds. In general, European *(Bos taurus)* dairy and beef breeds attain puberty earlier than do those of Zebu origin *(Bos indicus)*. Breed of sire and dam alters age at puberty, and crossbreeding reduces age at puberty in heifers.

Although timing of puberty is altered by many factors, as discussed later, age at puberty is influenced by selection. Breeds selected for milk production as well as for size (dairy and beef breeds) reach puberty earlier than do breeds of similar mature size and retail product growth potential but not selected for milk yield. Inbreeding delays puberty in heifers by retarding rate of growth. Estimates of heritability for age at puberty range from 0.41 to 0.64, two- to threefold higher than the estimates of heritability of milk production. With such relatively high heritabilities, practices of selection for earlier puberty in genotypes with traditionally later sexual development could prove beneficial, particularly in some beef breeds in which puberty often is delayed. Heifers sired by bulls with large scrotal circumferences reach puberty at younger ages and rebreed sooner after their first parturition than do heifers sired by bulls with small scrotal size. Studies also have demonstrated that heifers that are heavier at 6 months of age (before puberty) reach puberty at younger ages and also are heavier at calving. Although beef heifers that are heavier at weaning reach puberty at younger ages, these same heifers experience longer intervals to estrus after their first parturition than do their lighter-weight contemporaries.

Season

Although cattle are not considered to be seasonal breeders, evidence indicates that season of birth may alter timing of puberty. When heifers were fed similarly in controlled environmental chambers, those born in the autumn reached puberty earlier than those born in the spring.[2] Heifers born late within a spring calving season, however, were younger and lighter at puberty, probably because of improved forage quality and its effects on milk availability from their dams and digestibility of forages obtained by their grazing.

Body Weight and Nutrition

Although age and weight are correlated somewhat with the onset of sexual maturity, body weight is the major factor affecting age at puberty in cattle. Generally, the onset of puberty occurs when the heifer reaches approximately 40% to 50% of her mature weight. Unintended pregnancies may be initiated in heifers younger than 6 months of age when they are maintained in the presence of breeding bulls. Initiation of puberty normally occurs in yearling heifers, but age at puberty may range from 4 months to more than 2 years. Puberty is delayed in heifers that are maintained on low planes of nutrition during the prepubertal growth phase. Onset of puberty is dictated by body weight for the breed. Although age at puberty is influenced by nutritional factors, body weight at puberty is unaffected by nutrition. Correlations between gain in body weight and age at puberty indicate that increased growth rate of heifers results in younger age at puberty. In short, heifers on various planes of nutrition will reach puberty at different ages, but at similar relative stages of physical development. Furthermore, subsequent adequate weight gains are required once heifers reach puberty to ensure that they continue to have normal estrous cycles.

Underfeeding or overfeeding heifers has significant consequences on their development. Underfeeding of growing heifers may result in delayed puberty, subnormal conception rates, underdeveloped mammary glands, and greater incidence of calving problems.[3] Overfeeding often results in weak expression of estrus, subnormal conception rates, high embryonic mortality, decreased mammary gland development, and reduced milk production.[3] Dairy heifers fed 115% of all 1989 National Research Council nutrient requirements for heifers to gain 0.7 kg/day from 3 to 24 months of age were younger at puberty, were heavier at calving, had larger heart girth, were longer, were not excessively fat at calving, and calved at younger ages. Nutrient requirements suggested by the National Research Council should serve as minimum guidelines.

Social Rearing Environment

In some feral mammalian species, the physical presence of the male decreases age at puberty in contemporary females. Presence of mature bulls and adult cycling cows has altered age at puberty of heifers in some but not all studies. Increased proportions of prepubertal beef heifers treated oronasally with bull urine reached puberty earlier than water-treated controls.[3]

Endocrine Mechanisms

The events necessary to initiate the first pubertal estrus occur by an organized and interdependent cascade of changes in the central nervous system, hypothalamus, pituitary, and ovaries during maturation.[4] These events are summarized in Figure 35-1. As early as 4 months of age, the pituitary is capable of releasing luteinizing hormone (LH) in response to injections of gonadotropin-releasing hormone (GnRH). Likewise, the ovaries respond to exogenous and endogenous (GnRH-induced) gonadotropins by increasing the production of ovarian steroids.[4]

Early ovarian follicular development is evident. In fact, intrarectal ultrasonography shows that groups of follicles develop in regular repeating patterns (waves), beginning as early as 2 weeks of age, similar to what is observed in adult females during the estrous cycle and pregnancy[4] (see discussion of follicular dynamics below). The largest follicle, or dominant follicle, within each follicular wave of developing follicles progressively increases in maximal diameter from 2 to 34 weeks of age, parallel to increasing numbers of follicles detected in each follicular wave.[4] The combined evidence of early established pituitary and ovarian competence suggests that hypothalamic inactivity (lack of GnRH pulses secreted into the hypophyseal portal vessels) is responsible for maintaining the prepubertal status. The mechanism by which the hypothalamus remains relatively inactive has not been elucidated in cattle. Maturation of the GnRH pulse system (pulse generator) in the hypothalamus may necessitate other central nervous system involvement. Increased hypothalamic turnover and decreased sensitivity to various neurotransmitters occur in rodents as puberty is imminent. Specifically, increased turnover of norepinephrine and dopamine normally accompanies pulses of LH in the rat. Endogenous opioids may be involved in inhibiting the requisite LH pulse frequency, because administering an opioid receptor antagonist (naloxone) to 4-week-old heifers induced LH pulses, with the response decreasing as heifers approached 32 weeks of age.[4]

Evidence in ewes and heifers suggests that the prepubertal GnRH pulse generator is highly sensitive to the negative feedback of ovarian estrogens, such that estradiol inhibits secretory pulses of LH and probably those of GnRH as well (see Fig. 35-1). This phenomenon was described earlier in rats and is known as the gonadostat theory of puberty.[4] This theory suggests that the threshold to the negative feedback of estrogen increases as puberty approaches, accounting for a decreased sensitivity of the hypothalamus to the negative feedback of estrogen. Thus, the inhibitory mechanism modulating pulsatile release of GnRH and LH decreases, resulting in an increasing frequency of pulses, which normally are observed as puberty approaches (see Fig. 35-1). The pulse frequency of LH gradually increases between day 126 and day 14 before puberty, whereas concentrations of estradiol receptors within the hypothalamus decline.

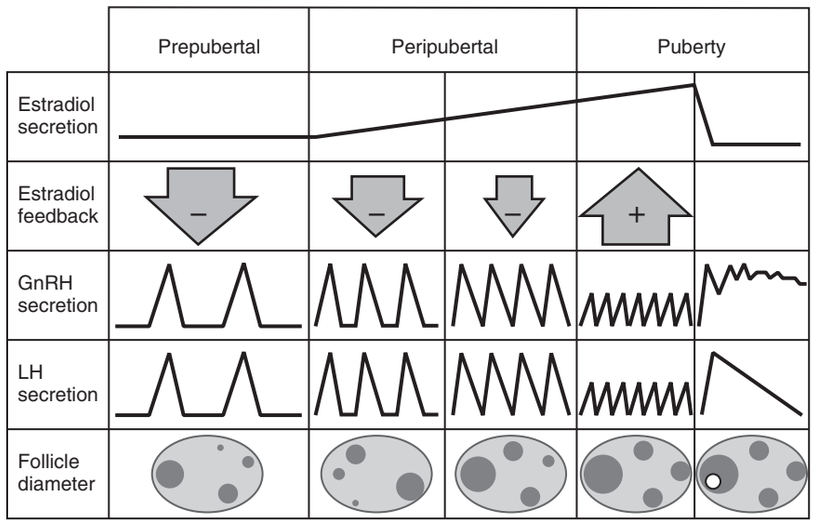

Fig. 35-1 Conceptual model for endocrine changes responsible for the onset of puberty in heifers. The peripubertal period includes approximately the 50 days preceding puberty in heifers. The *sign within the arrows* representing estradiol feedback indicates negative (–) or positive (+) feedback on secretion of luteinizing hormone (LH), and *width of the arrows* indicates relative degree of negative feedback. Secretion of gonadotropin-releasing hormone (GnRH) is highly sensitive to estradiol negative feedback during the prepubertal period. As the peripubertal period and the associated decline in estradiol negative feedback begins, secretion of GnRH, and hence LH, increases resulting in increased growth and estradiol secretion by dominant ovarian follicles. As a result of the progressive decline in estradiol negative feedback and increase in LH pulse secretion during the peripubertal period, estradiol eventually attains concentrations sufficient to induce the pubertal preovulatory surge of LH. (From Day ML, Anderson LH: Current concepts on the control of puberty of cattle. *J Anim Sci* 1998;76:1 Suppl3.)

Endogenous LH pulses occur at a frequency of one to four per 24 hours during the prepubertal period. As during the estrous cycle, the frequency of LH pulses sufficient to "drive" follicular maturation to ovulation must increase to approximately one pulse per hour. For 3 weeks before puberty, blood concentrations of progesterone seldom rise above 0.5 ng/ml (indicative of significant luteal tissue in the ovaries and little or no negative feedback of progesterone on LH secretion). Therefore, the frequency of LH pulses seems to be insufficient to support follicular maturation and ovulation during this period.[4]

The transition into puberty occurs for 2 to 4 weeks before first ovulation. This transient stage is characterized by an increased frequency of LH pulses and ovulation, as determined by a significant increase in blood concentrations of progesterone. In most cases, heifers in this transitional period exhibit one or two short-lived (duration of 2 to 5 days) elevations in blood progesterone of lower magnitude than what is observed in the normal estrous cycle. These transient increases in progesterone are preceded by LH pulses, the source of which may be luteinized ovarian follicles, and may be involved in preparing the uterus for the possibility of pregnancy and/or the establishment of normal patterns of GnRH and LH pulses characteristic of cycling females. The first major preovulatory LH surge occurs only after earlier prepubertal rises in progesterone, with a behavioral estrus observed in some heifers at this time. Puberty has been induced by administering a progestin as an ear implant containing norgestomet for 9 days,[3] in addition to an intramuscular injection containing both norgestomet and estradiol valerate, or as a progesterone-releasing intravaginal insert. Similar success has been reported when melengestrol acetate was fed for 7 to 16 days.[3] The effectiveness of these treatments depends on attainment of adequate body weight in heifers, characteristic of that necessary at normal puberty for their genotype.

PHYSIOLOGY OF THE ESTROUS CYCLE

General Traits

Once puberty occurs, estrous cycles generally continue unabated unless pregnancy is established or nutritional conditions are limited severely. Estrous cycles typically are 3 weeks in duration but normally may range from 17 to 25 days. The period of estrus may range from 2 to 50 hours in duration but averages 12 to 18 hours under most conditions. Ovulation occurs approximately 24 to 30 hours after the onset of estrus, with the first signs of estrus usually coinciding with the beginning of the preovulatory surge of LH and follicle-stimulating hormone (FSH). Durations of estrous cycles are often 1 to 2 days shorter in heifers than in cows. High seasonal temperatures do not seem to alter duration of cycles but may reduce the duration of estrus and decrease blood flow to the reproductive tract and may alter concentrations of various reproductive hormones.[2]

The estrous cycle is divided into two distinct phases—follicular (day 19 until estrus occurs) and luteal (days 1 to 18)—or into four stages—estrus (day 0), metestrus (days 1 to 3), diestrus (days 4 to 18), and proestrus (day 19 until behavioral estrus occurs). Estrus is characterized by sexual receptivity of the female (standing behavior) to a bull or to mounting activity by other females, in addition to follicular growth in preparation for ovulation. Metestrus is characterized by final follicular maturation and ovulation, formation of the early corpus luteum, and its subsequent ability to secrete progesterone. Once significant concentrations of progesterone are observed in peripheral blood, the luteal phase or period of diestrus begins and continues until the corpus luteum starts to regress at the onset of luteolysis. As concentrations of progesterone in blood begin to decline rapidly with the demise of the corpus luteum, the follicular phase is initiated, leading to the selection and accelerated growth of another ovulatory follicle.

Some important differences in these basic traits of estrus have been observed between *Bos taurus* and *Bos indicus* breeds. Specifically, *Bos indicus* females are reported to have periods of estrus of shorter duration, shorter intervals from the onset of estrus to ovulation, reduced magnitude of the preovulatory concentration of the LH surge, smaller corpora lutea, and lower luteal-phase concentrations of progesterone.[1,3]

Endocrine Patterns

For purposes of describing the interactions of hormones secreted by the hypothalamus, pituitary, ovaries, and uterus that are involved in the orchestration of sexual behavior and normal endocrinology of the estrous cycle, the cycle can be divided into three endocrine periods: (1) pregonadotropin surge, (2) postgonadotropin surge, and (3) luteal phase.[5]

Pregonadotropin Surge

The period of the pregonadotropin surge begins with the demise of the corpus luteum and ends with the preovulatory surge of LH and FSH, which initiate ovulation of a mature follicle 24 to 30 hours later (Fig. 35-2). Concentrations of progesterone rapidly decline as those of estradiol begin to increase concurrently with the accelerated growth of the preovulatory follicle. As progesterone declines, the baseline concentration of LH increases,[5] the frequency of LH pulses increases from about one pulse every 4 to 6 hours to one pulse every hour, but the amplitude of LH pulses declines[5] (all because of reduced negative feedback by progesterone on the hypothalamus and pituitary). This change in LH secretion, which coincides with a parallel pulsing of estradiol secreted into the venous effluent of the ovary bearing the preovulatory follicle, can be monitored in jugular blood by frequently collected blood samples (see Fig. 35-2). Although follicular growth may be stimulated by FSH alone, a combination of LH and FSH has been shown to induce maximal synthesis of estradiol in vitro.[6] Thus, both gonadotropins influence follicular development and subsequent secretion of estradiol. Estradiol appears to exert inhibitory effects on FSH release, because when it is administered to ovariectomized heifers, FSH is reduced to precastration concentrations. Furthermore, when titers of estradiol are

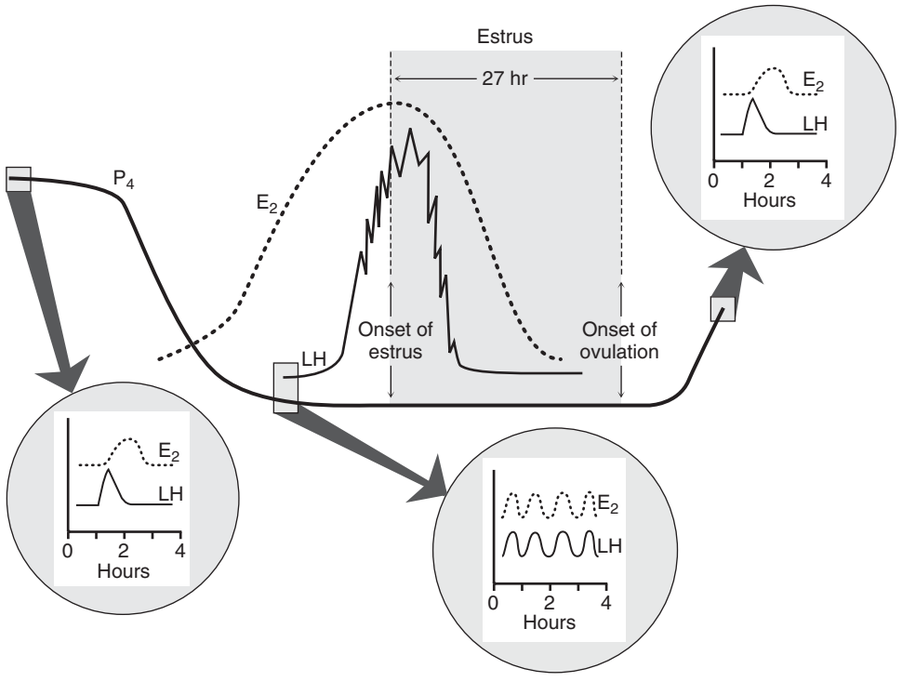

Fig. 35-2 Concentrations of progesterone (P_4), estradiol (E_2), and luteinizing hormone (LH) during the pre- and postgonadotropin surge period of the bovine estrous cycle. During diestrus (because of high concentrations of progesterone secreted by the corpus luteum), the frequency of LH pulses in jugular blood is low (approximately one per every 4 to 6 hours) and pulse amplitude is high, similar to that of estradiol in the ovarian venous effluent. As the corpus luteum dies and progesterone declines to baseline concentrations, the pulse frequency of LH increases to approximately one per hour (decreased amplitude) followed by correlated secretion of estradiol. The LH surge results from increased LH pulse frequency sufficient to sustain a peak (surge) of 8 to 12 hours induced by maximal titers of estradiol. These two events are coincident with the onset of estrus, which is followed by ovulation in approximately 27 hours.

at their peak just before the preovulatory surge, concentrations of FSH are lowest.[5]

A carefully orchestrated cascade of increasing LH pulses and elevated titers of estradiol eventually culminates in the onset of estrus, with estradiol triggering the initiation of both estrous behavior and the preovulatory surge of LH (see Fig. 35-2). Estradiol apparently triggers more frequent pulsatile GnRH secretion by the hypothalamus and enhanced pituitary responsiveness to each priming GnRH pulse. Blocking the rise in estradiol by chemical or immunologic means can block the occurrence of the gonadotropin surge.[5] Likewise, concentrations of progesterone must be low before estradiol can induce the gonadotropin surge, because administering progesterone during the period before the surge can block it. The elevation of LH and FSH (surge) usually endures for 8 to 10 hours. Termination of the surge is due to refractoriness of the pituitary to GnRH (decreased concentration [down-regulation] of pituitary GnRH receptors) and depletion of the releasable pool of pituitary gonadotropin. This hypothesis is substantiated by two observations: (1) When heifers are ovariectomized shortly after the onset of estrus and the preovulatory surge, a delay in the classic postcastration rise in LH is observed, and (2) injections of GnRH within 12 to 16 hours of the onset of the surge result in the release of only marginal amounts of gonadotropin.

Postgonadotropin Surge

The postgonadotropin surge period is characterized by declining titers of estradiol in blood, after their peak at the onset of estrus, as follicular luteinization ensues (Fig. 35-3). The process of ovulation has been likened to mechanisms associated with an inflammatory reaction.[7] The process of follicular rupture is regulated by a host of intrafollicular mediators. For example, ovulation can be blocked by intrafollicular administration of various prostaglandin enzyme inhibitors or antiserum to various prostaglandins or their regulatory enzymes.[5] Inter–theca cell capillaries and the basement membrane of the follicle are disturbed at ovulation, accounting for some hemorrhage, and thus allowing both capillaries and theca cells to pervade the ruptured follicle. Follicular content of estradiol declines rapidly as progesterone content begins to increase, because both theca and granulosa cells differentiate into luteal cells (luteinize) that form the corpus luteum. Theca cells appear to develop into smaller-diameter cells, known as small luteal cells, and the granulosa cells become large luteal cells as the corpus luteum develops.[8]

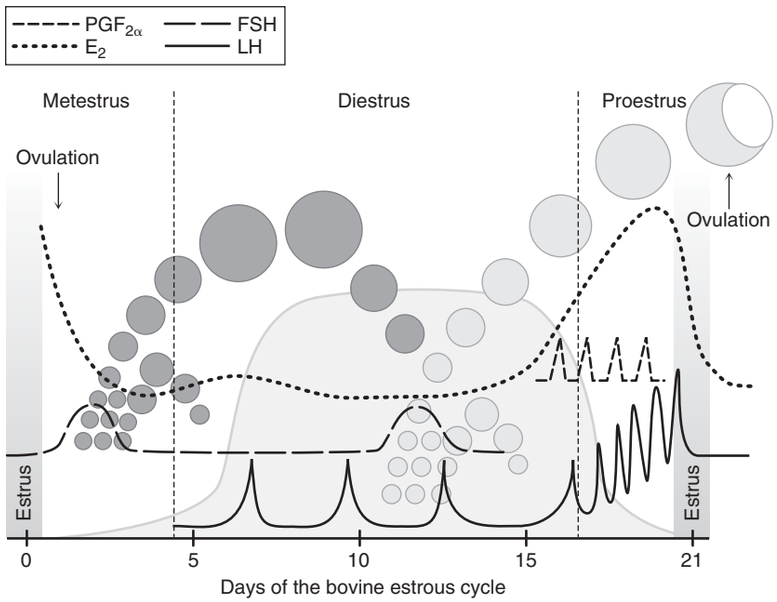

Fig. 35-3 Diameters of two dominant follicles and their respective subordinate follicles from each of two cohorts of follicular waves during the bovine estrous cycle. Peak concentrations of follicle-stimulating hormone (FSH) are detected 1 to 2 days before the recruitment of each follicular wave. The first peak is nearly coincident with ovulation during metestrus. During diestrus, when elevated concentrations of progesterone (P_4) are secreted by the corpus luteum, pulses of luteinizing hormone (LH) are sufficient to allow selection of dominant follicles but insufficient to allow maturation of a dominant follicle until progesterone returns to basal concentrations after spontaneous luteolysis (increased uterine-secreted prostaglandin $F_{2\alpha}$ [$PGF_{2\alpha}$]) or in response to exogenous injections of $PGF_{2\alpha}$. After luteolysis, during proestrus, LH pulse frequency and estradiol (E_2) increase sufficiently to induce behavioral estrus and the LH surge. (Adapted from Thatcher WW: *Fundamentals of dairy reproduction. The 100-day contract: Fundamentals for achieving reproductive efficiency CD-ROM.* Pharmacia Animal Health, Kalamazoo, MI.)

The remainder of what is called metestrus (days 1 to 3) is characterized by low blood concentrations of LH, estradiol, and progesterone (see Fig. 35-3). A secondary rise in FSH of lower magnitude than that observed during the surge often is reported just before ovulation, or about 24 to 30 hours after the onset of the preovulatory surge. This secondary rise may be a consequence of the loss of follicle-derived inhibin production during the ovulatory process. This increase in FSH probably is critical to the recruitment of the first wave of antral follicles that becomes visible by intrarectal ultrasonography during early metestrus[11] (see Fig. 35-3; see also subsequent section on follicular dynamics).

Luteal Phase

The luteal phase (days 4 to 18) begins when the corpus luteum secretes significant concentrations of progesterone, which generally exceed 1 ng/ml by day 4 or 5 of the cycle (see Fig. 35-3). As progesterone establishes itself again as the dominant negative feedback hormone of the luteal phase, patterns of LH secretion are modified once again to a lower frequency (one pulse every 4 to 6 hours) but with higher-amplitude pulses. Progesterone reaches maximal concentrations by days 8 to 10, which is nearly coincident with the maximum weight of the developing corpus luteum. Both cell types of the corpus luteum secrete progesterone, but the small luteal cells seem to have nearly all of the LH receptors and are six times more responsive to LH in vitro than the large luteal cells in terms of progesterone secretion.[8] The small luteal cells represent about 20% of the total luteal cell population and contribute approximately 15% of the progesterone secreted by the corpus luteum, whereas the remainder is derived from the large luteal cells. The large cells, however, have nearly all of the receptors for prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Concentrations and affinity of highly specific $PGF_{2\alpha}$ receptors in the bovine corpus luteum are similar on days 2, 4, 6, and 10 of the estrous cycle, failing to explain the lack of luteolytic response of the corpus luteum to $PGF_{2\alpha}$ before day 5 or 6 of the cycle. Large luteal cells also produce neurophysin and oxytocin, identical to those produced in the hypothalamus and stored in the posterior pituitary.[8] Early in pregnancy, about 20 days after conception, the original large cells disappear, leaving the small luteal cells, some of which expand into larger-diameter luteal cells in the corpus luteum of pregnancy.[8] The major luteotropin in cattle is thought to be LH, because if antiserum to LH is administered during the luteal phase, regression of the corpus luteum ensues.

Marked pulses of $PGF_{2\alpha}$ secreted by the uterus, in absence of a viable embryo, cause regression or death of the corpus luteum, thereby ending the luteal phase (see Fig. 35-3). After about 14 days under the influence of progesterone secretion, the endometrium secretes pulses of $PGF_{2\alpha}$, each lasting about 6 hours, for a total of approximately 36 hours. Prostaglandin $F_{2\alpha}$ causes an immediate decline of progesterone and oxytocin to basal concentrations within 6 to 10 hours in the peripheral circulation and in the content of the corpus luteum. Uterine involvement in the process of luteolysis is supported by the prolonged maintenance of the corpus luteum when the uterus is removed during the luteal phase. Furthermore, cattle with a congenitally absent uterine horn that is ipsilateral to the ovary bearing the corpus luteum have prolonged cycles with an extended luteal lifespan. Such observations, as well as surgical preparations in sheep, have provided evidence of a local utero-ovarian control of luteal lifespan. Current observations suggest that $PGF_{2\alpha}$ produced by the uterine horn adjacent to the ovary bearing the corpus luteum is transferred from the uterine venous effluent into the ovarian artery via a countercurrent transfer mechanism.

Some controversy remains concerning the mechanism by which $PGF_{2\alpha}$ induces luteolysis. Luteolytic effects of $PGF_{2\alpha}$ are thought to occur in part through its direct inter-

vention with luteal cells and, indirectly, via a reduction in luteal blood flow. Although $PGF_{2\alpha}$ has vasoconstrictive properties, it has not been determined whether the marked decrease in ovarian and luteal blood flow that occurs in spontaneous or $PGF_{2\alpha}$-induced luteolysis is a cause or consequence of luteolysis. Recent evidence indicates that luteal oxytocin is involved in luteolysis, probably through its ability to elaborate more secretion of $PGF_{2\alpha}$ from the endometrium as the corpus luteum begins to regress and oxytocin is released during luteal demise. Oxytocin given exogenously on days 5 to 8 of the cycle causes regression of the corpus luteum only if the uterus is present.[5] Estradiol produced by the dominant large follicle late in the cycle is thought to initiate the process of luteolysis by inducing production of uterine $PGF_{2\alpha}$. Estradiol induces uterine endometrial receptors for oxytocin, which, when occupied by oxytocin, activate phospholipase A_2 and release arachidonic acid and the arachidonic acid cascade, leading to production of uterine $PGF_{2\alpha}$ and eventual luteolysis. Administration of exogenous estrogens initiates luteolysis and can stimulate uterine production of $PGF_{2\alpha}$. Conversely, removal of ovarian follicles after day 10 of the cycle will extend luteal function for several days beyond the duration of a normal cycle. Therefore, the positive feedback cascade of oxytocin from the corpus luteum to the uterus and of $PGF_{2\alpha}$ from the uterus to the corpus luteum probably serves as a fail-safe mechanism to ensure luteolysis. The process of luteolysis and actions of $PGF_{2\alpha}$ can be monitored by measuring a major blood serum-stable metabolite of $PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (PGFM). Luteolytic pulses of $PGF_{2\alpha}$ (peripheral PGFM) are observed with concurrently detectable increases in estradiol and declining concentrations of progesterone (see Fig. 35-3).

Follicular Dynamics

Waves of follicular growth develop throughout the bovine estrous cycle. In each cycle, either two, three, or four waves of follicular growth can occur, with two waves being most common in adult cows.[6,9] An example of one cow with two follicular waves is illustrated in Figure 35-3. Each wave consists of a cohort of follicles that begin to increase in diameter from their original 1- to 2-mm size (recruitment), the largest of which becomes the dominant follicle by continuing to grow (selection), whereas others degenerate and undergo atresia.[9] Once dominant, the dominant follicle undergoes three phases of development: growth (increasing diameter); stasis (little change in diameter); and regression (decreasing diameter [atresia]). The first wave consistently begins around day 1 of the cycle. The second, third, and fourth waves begin at more variable times, with more waves occurring in cows with longer estrous cycles. Any dominant follicle can mature and ovulate, if the corpus luteum is forced to regress by exogenous injections of $PGF_{2\alpha}$ at the appropriate time. In other words, if $PGF_{2\alpha}$ were given and the corpus luteum regressed during the growing or early static phase of the dominant follicle, it could proceed to ovulation. Otherwise, if the dominant follicle has begun to become atretic, a new dominant follicle will develop from a new cohort of follicles, accounting for a longer follicular phase.[9] In retrospect, atretic follicles can be identified histologically by the presence of pyknotic nuclei in the granulosa cell layer or by the decreased ratio of estrogen to progesterone in the follicular fluid.

Emergence of each follicular wave is initiated by an increase in FSH[9] (see Fig. 35-3). The first wave is initiated by the secondary rise in FSH that follows its preovulatory surge. All other waves are initiated by detectable transient increases in FSH that precede the ultrasonographic appearance of each wave by 1 to 2 days. Increased estradiol in blood is observed shortly thereafter, as the largest follicle becomes dominant and estrogenic.[9] The largest follicle apparently dominates its subordinate cohort of follicles by producing various substances, including inhibin. Because of this orchestrated regular occurrence of follicular waves, at least one dominant follicle (codominance of two large follicles can occur) is always present in the pair of ovaries at any stage of the cycle. This phenomenon guarantees great difficulty in accurate prediction of stage of the cycle or impending estrus by palpation of the ovaries alone, even when a palpable corpus luteum is identified. Accurate diagnosis of a functional corpus luteum by palpation per rectum is difficult. Studies in which the presence or absence of functional luteal tissue was validated by concurrent measurement of progesterone in serum indicated that when a corpus luteum was identified by palpation, only 82% of the time was its presence validated by high concentrations of progesterone. When a corpus luteum was not palpated, progesterone concentrations were low 70% of the time, indicating that a corpus luteum was present but missed in 30% of the cases.

Follicular maturation follows coordinated actions of LH and FSH through receptors in theca and granulosa cells, respectively (Fig. 35-4). LH binds to theca cells and stimulates production of androgens, which subsequently diffuse through the basement membrane and into granulosa cells. Binding of FSH to granulosa cells increases aromatase activity, which converts androgens to estradiol. Increasing concentrations of estradiol and FSH also up-regulate receptors for LH in granulosa cells in maturing preovulatory follicles (<10 mm in diameter). Binding of LH and FSH by granulosa cells is necessary for regulating final follicular maturation and eventual ovulation in response to these hormones.[6] These two cell types and the associated gonadotropins are regulated by finely tuned mechanisms because estradiol also can inhibit the production of progesterone in both theca and granulosa cells, thus ensuring that androgens are produced via a parallel enzymatic pathway in both cell types (see Fig. 35-4). This system of regulation of theca and granulosa cells by LH and FSH often is referred to as the "two-cell, two-gonadotropin model."[6,8]

PREGNANCY

Pregnancy is defined as the period from fertilization until parturition. Gestation in cattle is approximately 280 days in duration, with a normal range of 270 to 292 days. Variability in the duration of pregnancy is influenced by fetal sex; number of fetuses; breed; genotype of the sire, dam, or fetus; plane of nutrition; and environmental factors.[1]

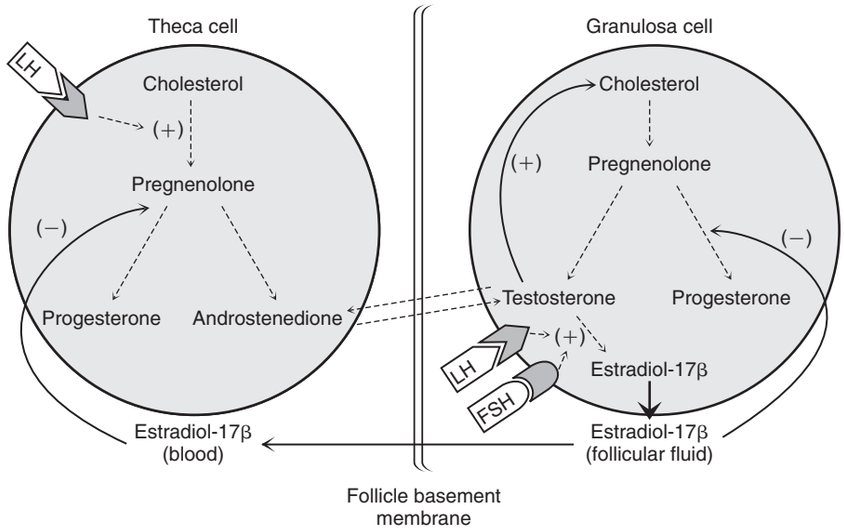

Fig. 35-4 Suggested two-cell, two-gonadotropin model for regulation of estradiol by preovulatory follicles. Theca cells provide androgen precursors in response to luteinizing hormone (LH) stimulation of cholesterol (derived mostly from low-density lipoprotein [LDL]-cholesterol) conversion to pregnenolone and androstenedione. Androstenedione crosses the basement membrane into the granulosa cells and is converted to testosterone in response to follicle-stimulating hormone (FSH) in small follicles and in dominant follicles in response to LH. Increased supply of testosterone leads to synthesis of estradiol, which is transferred into the follicular fluid or back across the basement membrane to increase blood concentrations of estradiol. Concentrations of estradiol inhibit the conversion of pregnenolone to progesterone in both follicle cell types.

Male fetuses are carried longer than female fetuses, whereas presence of twin fetuses results in shortened periods of gestation. Undernutrition and heat stress can shorten gestation, retard fetal growth, and result in weak calves.

If conception occurs at a given estrus, blood or milk concentrations of progesterone rise within 3 to 4 days, as in the normal cycle. Instead of declining at about day 17 or 18, however, they remain elevated for the duration of pregnancy (Fig. 35-5). The functionality of the corpus luteum must be spared (i.e., luteolysis must be prevented) to allow pregnancy to continue. Progesterone stimulates the glandular epithelium of the endometrium to proliferate and become secretory (histotroph or "uterine milk"), providing the only source of nutrients for the growing conceptus before placentation occurs and a minor portion even afterward. In addition, progesterone decreases uterine tone and myometrial contractility by increasing the threshold of sensitivity to various myometrial stimulants, thus allowing free expansion of the conceptus and placenta without expulsion from the uterus.

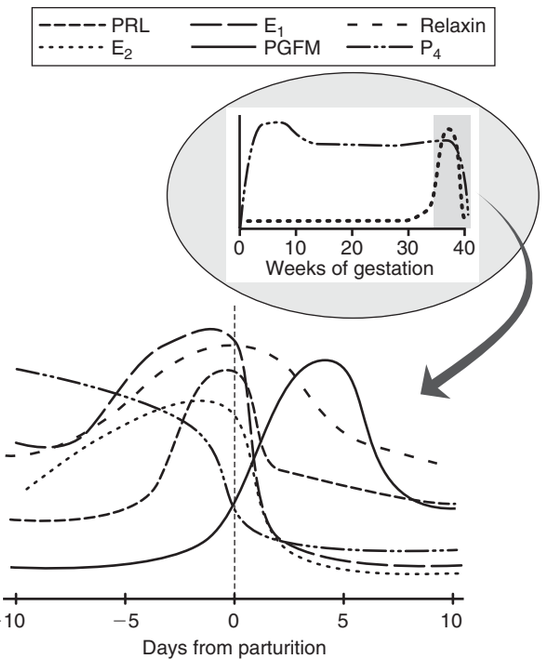

Fig. 35-5 **Upper panel,** Concentrations of progesterone (P_4) and estradiol (E_2) during gestation in the bovine. **Lower panel,** Relative acute changes in various hormones during late gestation before parturition are illustrated: P_4, E_2, estrone (E_1), relaxin, prolactin (PRL), and 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (PGFM).

Maternal Recognition of Pregnancy

At about day 16 or 17, the conceptus appears to signal its presence to its dam, an event known as **maternal recognition of pregnancy.**[10] The timing of this recognition was determined by an experiment in which embryos were transferred into the uterus at various times; it was

observed a viable embryo must be present in the uterus by day 16 or 17 to prevent luteal demise. If the embryo dies before day 16, then the cow will recycle in 18 to 24 days, as if conception never occurred. If the embryo dies after the demise of the corpus luteum is prevented, then the cow may have a delayed return to estrus (prolonged cycle). Up until this time, the hormones of the estrous cycle and pregnancy are effectively similar (see progesterone [P_4] and estradiol [E_2] in Figs. 35-3 and 35-4), and changes in the oviductal and uterine environments occur regardless of pregnancy status. Beyond this point, a viable conceptus in the uterus plays an active role in securing its survival there. Consequently, luteal maintenance, which is characteristic of pregnancy, reflects responses of the uterus and ovary to physiologic conditions initiated by one or more signals from the conceptus and its products.

Several hypotheses have been presented to explain the mechanism by which the embryo signals its presence and prevents luteolysis.[10] The embryo actively secretes a host of steroids, prostaglandins, and proteins into the lumen of the uterus beginning on day 13. Between then and day 16 or 17, noticeable conceptus-altered maternal physiology is not observed, but during this critical period, the normal cycle of events associated with luteal regression is modified to accommodate the existing pregnancy.

Embryos may produce an antiluteolytic substance that prevents luteolysis either directly or indirectly by inducing a uterine luteotropic substance. Prostaglandin E (PGE), when infused directly into the uterus beginning in the late luteal phase of the estrous cycle, has delayed luteolysis in some experiments. Furthermore, administering homogenates of day 10 to 12 bovine embryos into the uterine lumen also has delayed luteolysis.[10] Experiments have demonstrated that bovine blastocysts (obtained at days 13 to 19) produce progesterone, testosterone, estradiol, PGE, and $PGF_{2\alpha}$. A bovine-produced conceptus protein known as interferon-tau may signal changes in endometrial production of various prostaglandins in the presence of the embryo, thereby preventing luteolysis and maintaining the corpus luteum.[10] Therefore, the embryo probably produces a luteotropin that prolongs luteal life span. Around days 10 to 12 of the cycle or pregnancy, blood flow to the ovary bearing the corpus luteum increases markedly and remains elevated throughout pregnancy.[5,8]

Presence of the ovary bearing the corpus luteum is essential to maintain pregnancy in the cow until about day 200 of gestation. In one study, nearly all pregnant cows that were subjected to bilateral ovariectomy maintained their pregnancy, so long as their adrenal glands remained intact. Elevated concentrations of progesterone and placental estrogens during pregnancy inhibit pituitary LH secretion sufficiently to prevent a preovulatory surge of LH and ovulation (see Fig. 35-5). Continuing patterns of follicular waves into pregnancy have been reported, however.[9]

Hormonal Patterns

The fetoplacental unit secretes estrone (E_1) (see Fig. 35-5), which becomes sulfated and increases throughout pregnancy in conjunction with fetoplacental growth and development. Estrone and estradiol produced by the fetoplacental unit are conjugated immediately by the placentomes to prevent excessive estrogenic effects on maternal tissues. The conjugated estrogens (estrone sulfate) are pooled in the fluids of the chorioallantois and amnion of the placenta and in maternal circulation. A gradual increase in conjugated and free estrogens (1% to 10% of conjugated forms) occurs during pregnancy, beginning by day 60 of gestation. In addition, during the last 2 months of pregnancy, the placenta may be capable of maintaining pregnancy by its de novo synthesis of progesterone and estrogen.[1] Concentrations of estrogen increase markedly during the last 1 to 4 weeks of gestation (see Fig. 35-5). Other steroids also appear to be synthesized in the placenta but are depleted immediately after birth of the calf and expulsion of the placenta.

During the latter few weeks of gestation, various changes in ovarian and placental secretions occur (see Fig. 35-5). In addition to the changes in ovarian and placental steroids discussed above, concentrations of relaxin are increased by secretions of either the corpus luteum or the placenta to prepare the cervix, uterus, and pelvic ligaments for fetal expulsion. Similar changes in maternal concentrations of PGFM and prolactin (PRL). The peak in PRL occurs just before parturition, to initiate lactogenesis. If this peak is blocked by various drugs, milk yield after calving is suppressed severely.

PARTURITION

Pregnancy is terminated by the fetus once it is capable of surviving outside the uterus. The specific mechanisms differ slightly between species, but in general, the hormonal changes associated with parturition are those involved with final maturation of the fetus, termination of pregnancy, expansion of the birth canal, initiation of uterine contraction, maternal behavior, synthesis of milk, and the ability to eject milk.

The signal to initiate parturition appears to reside in the hypothalamic-pituitary axis of the fetus (Fig. 35-6).[11] Thus, surgical removal of the fetal pituitary or adrenal glands prolongs gestation. Adrenocorticotropic hormone (ACTH) administration to fetuses causes premature birth, as does injection of cortisol or its synthetic analogues to the dam. Increased cortical growth in fetal adrenal glands during late pregnancy occurs in the absence of noticeable elevations in fetal ACTH. Recent work indicates that production of prostanoids within the fetal brain influences fetal ACTH secretion and that induction of prostanoid biosynthesis near the end of gestation may be important in the process of parturition.[11] Fetal ACTH becomes elevated 1 to 2 days before birth, coincident with increases in corticosteroids, however.[11]

The target for the fetal cortisol, which results from ACTH release or uteroplacental transfer of cortisol given to the dam, is the placenta. The rise in fetal corticosteroids during the last month of gestation is responsible for activation of enzymes in cotyledons that increase the conversion of progesterone to estrogens or their precursors.[11] Such an increase in placental steroidal activity is evidenced by the dramatic rise in prepartum concentra-

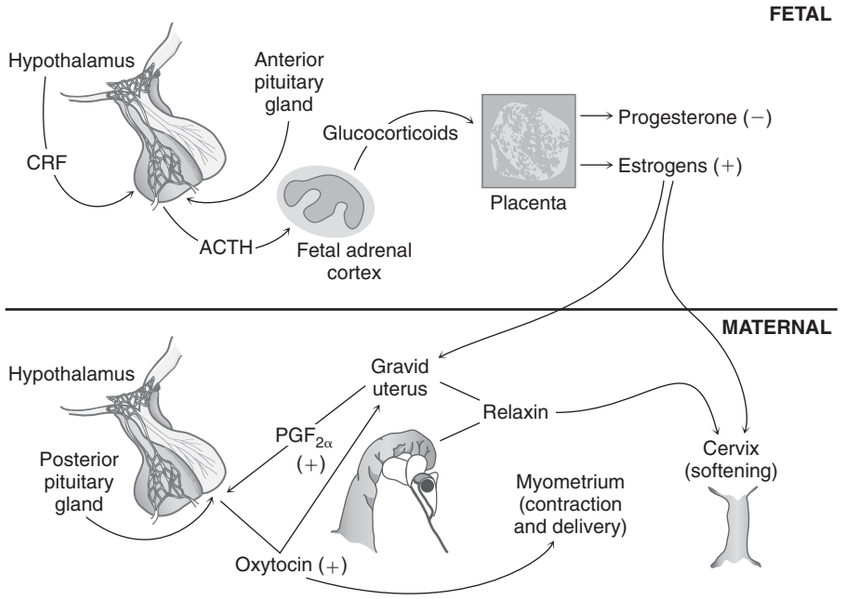

Fig. 35-6 A suggested sequence of fetal and maternal events leading to and associated with parturition in cattle. The time of parturition is controlled by maturation of the hypothalamic-pituitary-adrenal axis in the bovine fetus. Fetal corticotropin-releasing factor (CRF) is secreted from the hypothalamus and increases fetal adrenocorticotropic hormone (ACTH) from the anterior pituitary, which stimulates fetal glucocorticoid (cortisol) production by the adrenal cortex. In response to these events, (1) placenta production of progesterone declines (removal of progesterone block) and estrogen increases; (2) production of estrogen increases uterine secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), myometrial receptors for oxytocin, and placental or ovarian secretion of relaxin, which softens the cervix and relaxes pelvic ligaments to accommodate fetal expulsion; and (3) increased $PGF_{2\alpha}$ production stimulates maternal release of oxytocin from the posterior pituitary, which further stimulates $PGF_{2\alpha}$ production by the uterus and contractions of the myometrium with eventual delivery. (From Geisert RD: *Learning reproduction in farm animals CD-ROM.* Oklahoma State University, Stillwater.)

tions of estrogens, estrone sulfate, and other estrogen precursors (see Fig. 35-5). Estrogen and androgens secreted by the placenta augment activity of the fetal hypothalamic-pituitary-adrenal axis by increasing fetal ACTH secretion and by decreasing negative feedback sensitivity to cortisol.[11] In sheep, cortisol can stimulate the rate-limiting enzyme 17α-hydroxylase, causing the placenta to secrete predominantly estrogen, rather than progesterone.[11]

Progesterone in maternal blood declines gradually during the last week, before falling rapidly to baseline concentrations at parturition (see Fig. 35-5). The gradual decline in progesterone may be due partly to increased placental metabolism of progesterone to estrogens or their precursors. Estrogen, in turn, stimulates release of maternal $PGF_{2\alpha}$ from the uterine endometrium (see Fig. 35-5), resulting in the regression of the corpus luteum of pregnancy. Estrogen also induces receptors for oxytocin, thus preparing the uterus for parturition by oxytocin-induced contractions. Prostaglandin $F_{2\alpha}$ induces release of oxytocin from the posterior pituitary, and both hormones act as smooth muscle simulators to elaborate contraction of the myometrium and expel the fetus.[1,11] Furthermore, $PGF_{2\alpha}$, estrogen, and relaxin cause softening of the cervix and relaxation of the pelvic ligaments to facilitate birth.

Although much of the foregoing discussion has been proved experimentally, some details remain elusive at present and need to be documented.

PUERPERIUM AND POSTPARTUM PERIOD

After parturition, the cow enters a variable period of sexual quiescence or lactational anestrus before cyclic ovarian activity resumes. Although numerous factors probably interact to affect the duration of the postpartum anestrus, including breed and age of cow, milk yield, nutritional status, body condition, season, and presence of bulls, the presence (in beef cows) or absence (in dairy cows) of suckling seems to be the key inhibitor of estrous cycles.

Puerperium

Involution of the previously gravid uterus is another critical event that must occur during the early postpartum period.[12] The rate of involution is somewhat remarkable, because by 20 days after calving, tissue sloughing and hemorrhage have ceased, and the size of the uterus has been reduced by more than 80%. By 40 days, the uterus has completely involuted except for isolated pockets of

leukocytes. Involution, first estrus, and first ovulation are delayed in cows with periparturient problems such as dystocia, twinning, uterine infections, ovarian cysts, injury, or metabolic diseases such as ketosis, displaced abomasum, and milk fever.[13] Furthermore, all measures of reproductive efficiency are reduced in cows with periparturient problems compared with normal cows. Infertility or reproductive failure often is difficult to resolve because of its multiple causes.

Postpartum Period

Anestrus

Initiating early reestablishment of normal estrous cycles after calving is essential to allow adequate time for cows to be inseminated and maintain a 12- to 13-month calving interval. Numerous studies in dairy cows, based on observed estrous activity, ovulations, and resulting estrous cycles documented by repeated samples of milk or blood progesterone, indicated that nearly 95% resume cyclic ovarian activity by approximately 4 weeks after calving.[13] Recent studies have indicated that in well-managed dairy herds, the proportion of cows not cycling by the end of the volunteer waiting period (i.e., 60 days post partum) is approximately 20% to 25%.

Milking

Approximately 50% of dairy cows in one study[14] expressed estrus before this first ovulation (according to daily video recordings during the first 80 days after calving), whereas the herdsman observed estrous behavior in only 20%. Second ovulations (at about day 44 post partum) were preceded by estrous behavior in 95% of the dairy cows, whereas the herdsman observed such behavior in only 44%. By the third ovulation (at about day 64 post partum), 100% of the dairy cows were detected in estrus by video recordings, whereas the herdsman detected only 64%.

Suckling

In the beef cow, suckling prolongs anestrus, but its duration is shortened if calves are weaned at birth or suckling is limited.[13] Suckled beef cows that calve in the spring may not begin estrous cycles for 40 to 60 days. Early weaning of calves, however, usually initiates estrous cycles in most situations, regardless of body condition or nutritional status. Suckling per se (suckling and concurrent milk removal) is not essential to prolong anestrus, because cows maintained with their calves that were fitted with nose plates or muzzles so that they could not suckle had periods of anestrus equal to cows maintained with their normally suckling calves. An intact udder is not essential, so long as the calf is maintained with its dam and attempts to "suckle" after bottle feeding.[13] Suckling-induced hormones, such as prolactin, cortisol, and oxytocin, are increased in mastectomized cows when their own calves attempted to "suckle," analogous to those of udder-intact cows at the time of suckling by their own calves. In either case, concentrations of LH are low and pulses of LH are inhibited by suckling or attempted "suckling" events, so that anestrus is maintained.[13]

Partitioning of Available Nutrients and Body Condition

Combined effects of various metabolic changes and nutrient repartitioning, essential for lactogenesis and galactopoiesis in cattle, compete for nutrients that are essential to reinitiate estrous cycles after parturition. Nutrient intake, adequate availability of nutrients, diet composition, acute changes in body weight or body condition before or after calving, variously related metabolites, metabolic hormones, and overall energy balance are factors that correlate with the reestablishment of estrous cycles and may be indicators of intervals to first postpartum ovulation.

Metabolic functions are regulated in mammals to achieve two major purposes: self-survival and perpetuation of their species. **Homeostatic** mechanisms achieve the first purpose because they regulate the internal biochemical environment. **Homeorhetic** regulations achieve the second purpose by altering metabolic support to sustain growth, pregnancy, or lactation. The concept of homeorhesis accounts for how various hormonal mechanisms orchestrate coordinated changes in metabolic pathways in specific body tissues necessary to support an existing physiologic function such as lactation or pregnancy at the expense of other, less vital bodily functions. For example, when lactation begins, various priorities for nutrient utilization to support lactation are established. These priorities involve redirecting homeorhetic mechanisms that may be mediated by prolactin, somatotropin, insulin, insulin-like growth factor I (IGF-I), estradiol-17β, glucocorticoids, and other hormones.

The approximate ranking priority for use of available energy in ruminants is as follows: (1) basal metabolism; (2) activity; (3) growth; (4) energy reserves; (5) pregnancy; (6) lactation; (7) additional energy reserves; (8) estrous cycles and initiation of pregnancy; and (9) excess reserves.[13] In accordance with this prioritized use of available dietary nutrients, reinitiation of the estrous cycle and all of its associated components (i.e., gonadotropin secretion, ovarian follicular development, and ovulation) occurs only after greater-priority needs are met (i.e., maintenance, growth, lactation, and minimal energy reserves). Nutrient repartitioning for milk production and reproduction is the result of complex interactions among diet quantity and quality, nutrient reserves (body condition), and the demand for growth and metabolism.

Differences in management of milked and suckled cattle of various genotypes account for some of the differences in postpartum reproductive recrudescence, but as previously discussed, their lactational management is the major determinant. In general, if adequately fed, cows nursing a calf rarely experience a significant negative energy balance or extensive loss in body weight that occurs in full-fed, milked cows producing up to five times more milk. The effect of nutrition in suckled cows depends somewhat on whether nutrition is adequate before or after parturition. In general, prepartum nutrient status, as estimated by body condition at parturition, is more important than that after calving. The relationship between body condition at calving and the interval to first postpartum estrus in suckled beef cows is nonlinear. That is, the effects of poor body condition are greatest at

very low body condition scores (<4 on a 10-point body condition scoring system) and become less significant as body condition increases to a value of 7 or greater. When nutrition is adequate in suckled cows, milk yield has little effect on postpartum anestrus, but when nutrition is limiting, particularly for cows having genotypes for high milk production, increased milk yield delays first ovulation and estrus. In milked cows, such a relationship of interval to first estrus and body condition has not been established. The magnitude of nutrient deficit (negative energy balance), however, seems to be an important factor that inhibits first ovulation and has some relationship to the number of days to first ovulation.

Postpartum changes in energy balance generally are predictive of when first ovulation will occur in milked cows. In milked cows, the nadir of energy balance occurs during the first or second week after calving and recovers at a variable rate, with first ovulation occurring approximately 10 to 15 days after the nadir. Days to first ovulation are correlated positively ($r = 0.76$) with days to nadir of energy balance. Furthermore, because milk fat percentage is high near the nadir of energy balance, it may be a good indicator of the severity of nutrient deficit. During the enormous metabolic demands of high milk production in early lactation, major amounts of nutrients are required for mammary synthesis of lactose, proteins, and triglycerides in the mammary gland that cannot be met by dietary intake. Nevertheless, milked cows with greater dry matter intake, despite having a negative energy balance, produce more milk, lose less body weight, and ovulate earlier post partum than those with lower intakes. Cows with greater intakes also reach the nadir of energy balance earlier and experience a more severe, but shorter, period of negative energy balance, suggesting that when cows are more efficient in partitioning dietary and stored nutrients toward milk synthesis, they also are better able to recover ovarian cyclicity.

Hormonal and Follicular Changes

Blood FSH concentrations increase from prepartum concentrations in suckled and milked cows within 5 days and peak before the appearance of each follicular wave after calving. The first or second dominant follicle generally ovulates in milk dairy cows after LH pulse frequency increases. Concentrations of LH in plasma or serum increase gradually from low, nearly nondetectable levels at calving until about day 10, when some dairy cows are observed to begin pulsatile secretion of LH.[1,13] The frequency of the LH pulses (determined by the pulse frequency of GnRH) increases over a period of days (to about one pulse per hour) until ovulation occurs. An hourly pulse frequency may be necessary for initiation of sufficient LH release, so that final follicular growth can occur before first ovulation. An increase in IGF-I is coincident with increases in LH pulse frequency and earlier first postpartum ovulation.

After ovulation, the frequency of GnRH and LH pulses decreases to one every 4 to 6 hours. Onset of these episodic patterns is delayed in suckled cows, particularly in those intensively suckled by several calves or suckled by one calf, but in poor body condition or in negative energy balance.[13] As a result, many follicular waves occur, each with its dominant follicle, but without ovulation until LH pulse frequency increases sufficiently. Studies have demonstrated that pulses of GnRH every 1 to 2 hours, and hence small pulses of resulting LH, will induce estrous cycles in suckled and milked cows.[13] Part of this mechanism whereby LH pulses induce ovulation is related to the ability of the hypothalamic-pituitary axis to respond to estradiol that is elaborated from developing antral follicles. In the early postpartum period, milked cows respond earlier than suckled cows to this positive feedback effect of estradiol on LH secretion.[1,13] Not only does a temporal delay occur in the LH response to exogenous estradiol, but the magnitude of LH release in response to the same dose of estradiol increases as the postpartum period progresses.

Most current evidence suggests that the temporary inhibition of estrous cycles during the postpartum period is due to a block at the hypothalamic-pituitary level. Factors that influence the release of GnRH seem to be mediated within the brain or central nervous system by other hormones or endogenous opioid peptides.[13] The latter belongs to a family of endogenous hormones that mimic actions of the opiates, such as morphine or opium. Infusions of either of the opioid receptor antagonists naloxone and naltrexone increase concentrations of LH in milked and suckled cows, suggesting that endogenous opioid peptides limit LH secretion during the early postpartum period, when endogenous pulse frequencies of GnRH and LH are infrequent.

The pulse frequency of LH increases gradually until a preovulatory LH surge occurs to ovulate a mature graafian follicle. The first preovulatory surge of gonadotropins is followed, as expected, by a rise in progesterone in 3 to 4 days. In approximately 20% to 50% of dairy cows, however, this luteal phase is of short duration (<10 days), whereas in suckled cows weaned at about 30 to 40 days post partum or after their first ovulation, 90% will display a shortened estrous cycle (<12 days). This short cycle appears to be due to a premature release of uterine $PGF_{2\alpha}$, increased sensitivity of the luteal tissue to early luteolysis, and a higher concentration of uterine oxytocin receptors.[1]

Other Factors

Exposing cows suckled post partum to bulls decreases the period of anestrus. Season influences the length of anestrus in suckled cows. Although season may be modified by other factors such as suckling, nutrition, and genotype, truly seasonal effects are related to day length. Some evidence indicates that fall-calving primiparous (not multiparous) cows may have delayed intervals to first ovulation and estrus, unless supplemental lighting is given to provide 16 to 18 hours of total light per day.[2] The relationship of day length and prolactin suggested that the high concentrations of prolactin associated with milking and suckling may inhibit the onset of ovarian cycles. Evidence against this mechanism, however, includes the following findings: (1) Treatment with pharmacologic agents to reduce prolactin concentrations does not hasten the onset of ovarian cycles; (2) infusion of prolactin into cows fails to alter prolactin concentrations; and (3) concentrations of prolactin have

not been shown to be higher in suckled than in milked cows.

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CHAPTER 36

Estrus Detection

MICHAEL L. O'CONNOR

Faulty estrus detection is the most important problem in reproductive management confronting the herd manager and the veterinarian. For example, Barr[1] determined that the correlation between days lost because of missed heats and total days open was 0.92. Research using computerized simulation analysis clearly illustrated the economic advantage to improving the efficiency of estrus detection in dairy herds.[2] In this study, moderate improvement in the rate of estrus detection from 40% to 50%, coupled with a conception rate of 50%, increased income by $34 per cow per year. Further improvement to 60% estrus detection returned a total of $56 per cow per year. Although the costs to achieve such improvements were not considered in the analysis, the expenses associated with additional labor to more intense estrus detection, possible use of estrus detection aids, or an estrus synchronization program should result in a favorable net return per dollar invested. Earlier research reported a 4 to 1 return on investment to improve the rate of detection from 35% to 55%.[3] Failure to detect estrus or inaccurate heat detection results in an estimated annual loss of over $300 million to the dairy industry in the United States.[4] Poor estrus detection can be costly for a beef cattle cow-calf unit using artificial insemination for several reasons: (1) more conceptions occur late in the breeding season, reducing the potential value of calves because they are born late in the calving season; (2) cows bred late are likely to be bred late in subsequent years; and (3) more cows probably will be culled because they are not pregnant.

This chapter describes the characteristics of estrus, factors affecting estrous behavior, and methods to enhance estrus detection to include detection aids.

ESTROUS BEHAVIOR

Standing to be mounted is the most definitive sign of estrus in cows. During the period of **standing heat**, cows stand to be mounted by other cows. Cows that move away quickly when a mount is attempted are not in estrus. The average duration of a mount is approximately 2.5 seconds.[5] Considerable variation has been reported in

not been shown to be higher in suckled than in milked cows.

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ESTROUS BEHAVIOR

Standing to be mounted is the most definitive sign of estrus in cows. During the period of **standing heat**, cows stand to be mounted by other cows. Cows that move away quickly when a mount is attempted are not in estrus. The average duration of a mount is approximately 2.5 seconds.[5] Considerable variation has been reported in

the number of mounts received by an estrous cow. Using electronic pressure-sensitive mount detectors, Dransfield and co-workers determined the average (± SD) number of standing events for Holstein cows to be 8.5 ± 6.6 per cow.[6] The average duration of standing estrus, defined as the time from first mount to last standing event, ranged from 33 minutes to 35.8 hours, and the overall average for cows monitored in 17 commercial herds was 7.1 ± 5.4 hours. Standing behavior is critical because the onset of standing heat (estrus) is closely related to the time of ovulation. During the onset of estrus, estradiol (E_2) causes release of luteinizing hormone, initiating the process of ovulation. Using ultrasound examination to determine time of ovulation in relation to the onset of estrus based on electronic detection of estrus, the average interval from onset of estrus to ovulation was 27.6 ± 5.4 hours.[7]

Secondary signs of estrus include attempting to mount other cows, mucous discharge from the vulva, swelling and reddening of the vulva, bellowing, restlessness, trailing other cows, chin resting, sniffing the genitalia of the other cows, and lip curling. These signs may occur before, during, or after estrus and are not related to the time of ovulation. Such signs should be used as clues that cows are near estrus so that they can be watched more intensely for standing behavior. Figure 36-1 illustrates the relative changes in standing behavior and certain secondary signs before, during, and after estrus.

Estrus expression is maximized when the female has been previously exposed to progesterone (P_4), followed by a decrease in P_4 and an increase in E_2. The effect of E_2 for initiating behavioral signs of estrus appears to be "all-or-none." Once the threshold concentration of E_2 to induce estrus is achieved, additional E_2 does not further enhance the behavioral intensity of estrus. Furthermore, the inhibitory effect of high concentrations of P_4 on estrous behavior also is all-or-none.[8] Although E_2 and P_4 are considered the primary hormones involved in estrous behavior, the response of hypothalamic neurotransmitters to these steroids probably is the final signal controlling estrous behavior.

Estrus without subsequent ovulation and formation of a corpus luteum (CL) is estimated to occur in 60% of all peripubertal heifers. This is termed **nonpubertal estrus** and occurs early during the onset of puberty. By contrast, the condition of ovulation without estrus occurs toward the end of the postpartum anestrous period. This is termed **silent heat**, or silent ovulation, and generally occurs at the first postpartum ovulation in lactating cows. The incidence among dairy cows ranges from 50% to 94%.[8] The physiologic mechanisms responsible for this syndrome have not been resolved. One theory is that high concentration of estrogens present during late gestation and parturition induce a refractory state to the high concentrations of E_2 present at the first postpartum

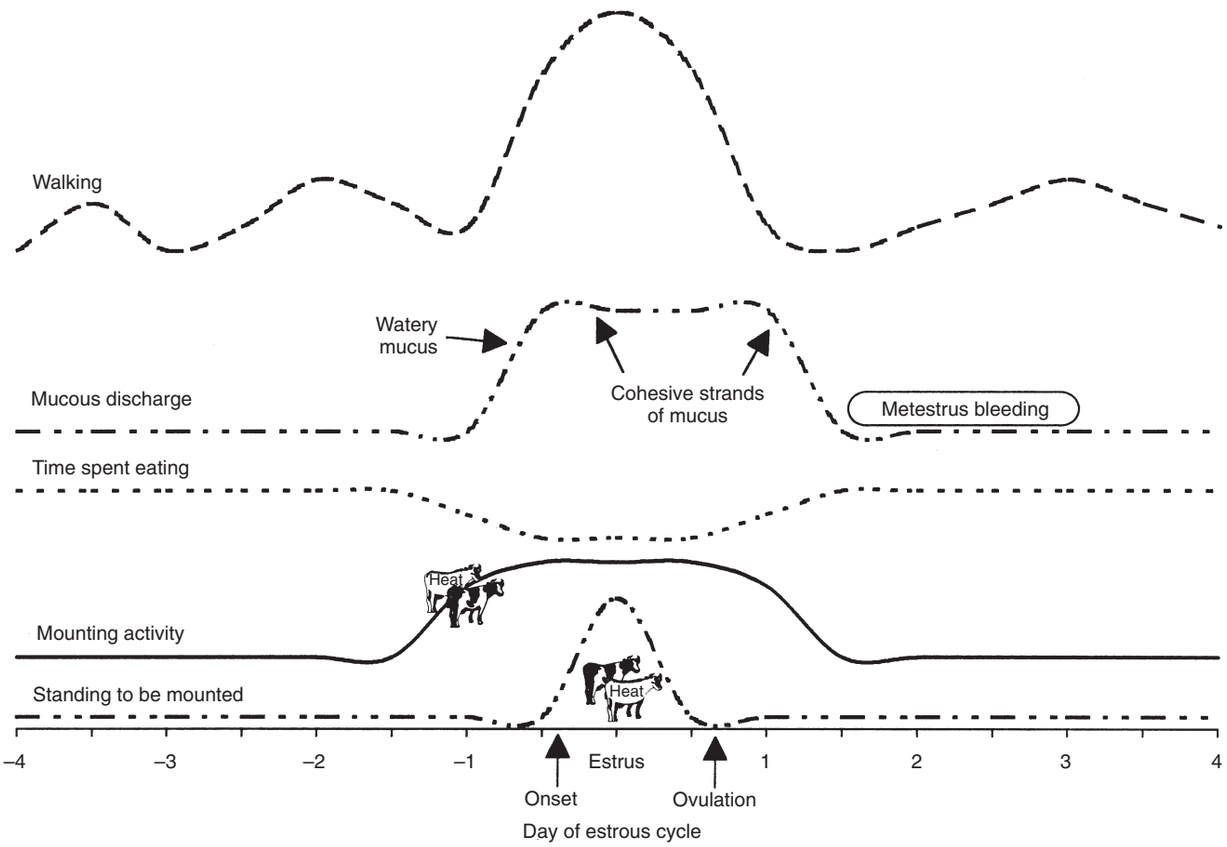

Fig. 36-1 Relative changes in standing behavior and secondary signs before, during, and after estrus.

ovulation. Consequently, estrus is not exhibited. P_4 released from the initial CL formed after the silent ovulation, however, overcomes the refractory state, allowing estrus to be expressed during the next ovulatory cycle.[8] A second possible explanation for the poor expression of estrus or silent ovulation is the low ratio of E_2 to P_4 concentrations. Vailes and co-workers showed that the estrogen-to-progesterone ratio caused a small but significant variation in mounting activity.[9] Behavioral traits that require active involvement of the cow (mounting and chin resting) do not appear to be suppressed by P_4 as much as receptive behaviors (mounts received, standing when mounted) are. Standing behavior, therefore, appears to be the behavior most sensitive to inhibition by P_4.[10]

Occasionally, pregnant cows exhibit signs of estrus. It is most frequently observed during middle to late gestation. Cows with ovarian follicular cysts have hormonal relationships similar to those in cows in estrus and may express estrus; however, most cows with ovarian cysts are anestrus. Some cows and most heifers have a bloody mucous discharge 1 to 3 days after estrus, but onset of such **metestrus bleeding** is quite variable. This discharge indicates that the cow was in estrus and does not mean that she failed to conceive.

EVALUATION OF ESTRUS DETECTION

It is important to periodically compare estrus detection efficiency with specific, realistic management goals. Complete and accurate records, including all dates of estrus, services, and pregnancy status information, are needed to calculate estrus detection efficiency. Most dairy record processing centers and herd management computer programs provide an estrus detection index. Other reproductive parameters, such as average days to first service and distribution of interestrual intervals, are useful in evaluating estrus detection. Most calculations of estrus detection efficiency or heat detection rate are based on the **voluntary waiting period** (VWP), which is the number of days after calving before which a dairy producer will not inseminate a cow, even if she is detected in heat.

Estrus detection efficiency is defined as the percentage of possible estrous periods that are observed during a specified period of time. Several methods have been developed to express the percentage of cows detected in estrus relative to the number of cows assumed or calculated to be in estrus. A review of this topic with equations and tables for determining the efficiency of estrus detection is available.[11] Computation of estrus detection efficiency and comparison among the various methods often are difficult because of differences in the time intervals used and in whether pregnant and open cows are included, and because of certain assumptions that may or may not be applicable. These assumptions include the following: (1) all cows are cycling by a certain day post partum; (2) reported estruses are in fact true estrous periods; (3) values used in the equations are accurate; and (4) the duration of all estrous cycles is 21 days, which is not the case with use of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The frequency of the use of $PGF_{2\alpha}$ and other synchronization programs to induce estrus and ovulation should be considered in evaluating any index of estrus detection efficiency.

Characteristics of Herds with Inefficient Estrus Detection

Using summary data from DHIA records or computer management programs or information from herd records such as days to first service and results from veterinary examinations, one can determine if the following characteristics of inefficient estrus detection exist.

- Very few estruses observed or recorded before first service
- Prolonged parturition to first service interval (which should be less than 21 days beyond the VWP)
- Excessive interval between services, especially when many intervals are multiples of a normal cycle (e.g., 42 days)
- Average interestrual interval greater than 35 days
- Greater than 25% of the cows routinely confirmed open at pregnancy examination 35 to 45 days after insemination
- Veterinary examinations confirming cows are cycling normally but estrous periods are not routinely detected
- Herd management computer programs or DHIA data routinely indicating heat detection rate less than 65%

Determining the Accuracy of Estrus Detection

Accuracy is defined as the percentage of estrous periods observed that are indeed true estrus. Frequently, management of herds with suboptimal reproductive performance is characterized by both inefficient and inaccurate estrus detection. Inaccuracies occur when cattle are inseminated at times other than true estrus. Examining the frequency distribution of interestrual intervals has been shown to be helpful in documenting estrus detection errors. Cows determined not to be in or near estrus at their first postpartum insemination on the basis of progesterone levels had abnormal intervals to the second service.[12] Twenty-nine percent and 20% of the intervals were between 11 and 14 days and 30 and 31 days after first service, respectively. Thus, cows incorrectly determined to be in estrus were in the midluteal phase when initially inseminated. Factors that contribute to errors in estrus detection include too much reliance on secondary signs instead of standing behavior to determine estrus status, unfamiliarity with correct signs of estrus by herd personnel, poor cow identification resulting in selection of cows to be inseminated that are not in estrus, and incorrect use or interpretation of estrus detection aids. The impact of all of these factors is magnified as herd size increases.

In addition to evaluating the distribution of interestrual intervals, milk progesterone testing can be a method of evaluating the accuracy of estrus detection. To document the variation in error rates of estrus detection

Table **36-1**

Error Rates for Estrus Detection in Cows Based on Milk Progesterone Concentration*

% of Cows within Herd with 1 ng/mL Progesterone Level at Insemination	No. of Herds within Each Category	Error Rate†
0	283	60.6%
<10	40	8.8%
10–19	102	21.8%
20	42	9.0%

*Data from Northeast dairy herds in which 5.1% of cows overall subsequently were found not to be in estrus.
† Percentage of herds classified as nonestrous.
Modified from Reimers TJ, Smith RD, Newman SK: Management factors affecting reproductive performance of dairy cows in the northeastern United States. *J Dairy Sci* 1985;68:963.

Table **36-2**

Estrual Activities on Dirt and on Concrete*

	MEAN NO. OF EVENTS	
Estrus Factor	Dirt	Concrete
Duration of estrus (h)	13.8	9.4
Activity during entire period of estrus		
Mounts	7.0	3.2
Stands	6.3	2.9
Activity during 30-min observation		
Mounts	3.7	2.5
Stands	3.8	2.7

*Means differ ($P < 0.01$) between locations.
Modified from Britt JH, Scott RG, Armstrong JD, et al: Determinants of estrous behavior in lactating Holstein cows. *J Dairy Sci* 1986;69:2195.

among selected Northeast dairy herds, milk samples were collected on the day of insemination and analyzed for progesterone using radioimmunoassay. Cows with progesterone concentrations greater than 1 ng/mL at the time of insemination were considered not to be in or near estrus. The proportion of herds with various rates of estrus detection errors is presented in Table 36-1. Although overall only 5.1% of the cows were not in or near estrus when inseminated, the percentage of cows classified as not in estrus ranged from 0% to 60% among herds. Greater than 30% of the herds had error rates in excess of 10%.[12] Sturman and co-workers, using thrice-weekly milk progesterone analysis, determined that approximately 19% of inseminations were made when cows were in mid-cycle or in early pregnancy.[13]

FACTORS AFFECTING ESTROUS BEHAVIOR

Factors related to environment, nutrition, herdmates, and condition of feet and legs dramatically affect estrous behavior.

Type of Housing

Any housing arrangement that allows cattle to interact throughout the day provides more opportunity for expression of mounting and standing behavior. In fact, tie stalls and stanchion barn housing are not appropriate for optimizing estrus detection unless cows are released on a regular basis for observation of estrous behavior. If cattle are not allowed to interact, the herd manager must use secondary signs to guess which cows are in estrus.

Using milk progesterone analysis by radioimmunoassay to determine the percentage of cows that probably were not in estrus at the time of insemination, researchers found a slight but significant increase in detection errors in free-stall herds, compared with herds with conventional housing.[12] In addition, the proportion of cows determined to be in or near estrus when inseminated and conception rates were not related to herd size. Although opportunity to observe estrus is greater when cows interact frequently in free-stall barns or loose housing, taking time to observe estrous behavior is essential no matter what type of housing in used.

Footing Surface

The composition of the footing surface where cows interact significantly affects the intensity of estrous behavior. Research conducted in North Carolina compared estrous activity in high-producing Holstein cows that were observed for estrus during 30 minutes on dirt and 30 minutes on grooved concrete. A summary of mounting and standing behavior from this study is presented in Table 36-2. Behavioral expression of estrus lasted longer for cows observed on dirt. Mounting and standing behavior were nearly doubled when cows were observed for estrus on dirt compared with concrete.[14]

Icy or slick concrete is always a problem in free-stall herds and concrete exercise lots, alleyways, and entrances to conventional barns. Concrete should be grooved to provide better traction. Moving cattle to a dirt lot or from one area to another not only provides a better surface but also provides added stimulation of moving cattle. Moving cattle enhances behavioral symptoms of estrus.

Foot and Leg Problems

Cows with sore feet or legs or poor structural conformation are less likely to exhibit mounting activity, or they may stand to be mounted when not in estrus because it is too painful to avoid being mounted. This is confirmed by the results from a British study involving 770 cows with nearly 1500 lactations: Lameness caused by specific lesions on the hoof was associated with a 7-day increase in days to first service and 11 more days open compared with herdmates without lameness.[15] These differences were greater for cows with sole lesions that developed between 36 and 70 days post partum, the time when cows should first be detected in estrus. For those cows, the

interval to first service and days open increased by 17 days and 30 days, respectively. Data from a study involving 837 cows of which 30% were diagnosed as lame, most with claw lesions, showed that the number of services per conception was significantly higher in lame cows than in healthy (nonlame) cows. The median time to conception for lame cows with claw lesions was 140 days, compared with 100 days for healthy cows.[16]

In a subsequent study, the relationship between lameness and the onset of cycling during the first 60 days of lactation was evaluated.[17] Using the new modified six-point locomotion scoring system, cows in a large commercial dairy were examined weekly during the first 35 days of lactation for diagnosis of lameness. In addition, blood samples were obtained to determine the concentrations of progesterone, which indicate the onset of ovarian activity. Cows classified as lame (score 4) were 3.5 times more likely to have delayed ovarian activity than nonlame cows. Furthermore, ketosis was found to be a factor that delayed cyclicity. It was suggested that lameness and ketosis might interact to delay ovarian activity. Lameness depresses dry matter intake, causing negative energy balance and production of ketone bodies. If cows are in pain from lameness, they spend less time eating and ruminating and more time lying down. Consequently, dry matter intake will be reduced, which potentially may delay the onset of cycling during early lactation.[6]

Influence of Herdmates

Herd managers must rely on open cycling herdmates to detect cows in estrus. Cows in midcycle, however, do not participate in mounting activity as frequently as do cows in proestrus or estrus. In a controlled study, estrous cows encountered cycling herdmates one on one for 10 minutes.[18] Total number of mounts by each herdmate was determined on day 10, day 15, day of estrus, and day 5 of the next cycle. Herdmates in midcycle (days 10 and 15) and on day 5 had one mount or less during the 10-minute observation period. When the herdmate herself was in estrus, however, the number of mounts increased significantly. Thus, taken as a group, cows in the luteal phase are poor estrus detectors. Because this phase accounts for 50% to 60% of the estrous cycle, which reduces the number of effective estrus-detecting herdmates, managers must rely on cows in or near estrus to detect other estrous cows. Additional research revealed that approximately 86% of the mounting among dairy heifers was by heifers in proestrus and estrus.[19]

In small herds, most of the herd may be pregnant at certain times, and the stage of the cycle of the few nonpregnant cows may be such that they are not effective estrus detectors. As more animals become pregnant, the number of potential estrus-detecting animals is reduced. The situation is similar for herds that freshen on a seasonal basis. After an intensive breeding period, when a high percentage of the herd is pregnant, it becomes increasingly difficult to identify the few open cycling cows in estrus. There simply may not be enough herdmates in the proper stage of the cycle to interact with an estrous cow. A third situation may occur frequently in large herds in which cows are grouped according to production. Generally, the lower-production group contains a high percentage of pregnant cows, and some open cycling cows may be included in this group. It becomes very difficult to detect the open cows in estrus because their herdmates are pregnant.

Table **36-3**

Number of Cows in Simultaneous Estrus and Mounting Activity

No. of Cows in Estrus*	NO. OF MOUNTS PER COW	
	Mean	Range
1	11.2	1–30
2	36.6	1–179
3	52.6	1–173
4 or more	49.8	1–130

*Per herd.
Modified from Hurnick JF, King GJ, Robertson HA: Estrous and related behavior in postpartum Holstein cows. *Appl Anim Ethol* 1975;2:55.

Estrous activity also is influenced by the number of herdmates in proestrus and estrus simultaneously. Canadian scientists found that the number of mounts increased significantly when two or more cows were in estrus simultaneously[20] (Table 36-3).

Variation in Mounting during the Day

It would be useful to develop an estrus detection strategy based on time periods when cows are most likely to express estrus. Nebel reviewed research using radiotelemetric systems to document the onset of estrus and mounting activity throughout the day.[21] Data collected over five years from a Virginia Tech dairy herd showed no differences for the onset of estrus across hourly intervals for heifers or cows. Management practices, however, affected the onset of estrus. When heifers on pasture were assembled for concentrate feeding, a peak of mounting activity was observed. Distribution of the onset of estrus for cows increased when cattle were moved in groups for milking and when moved to dirt lots. Similarly, in New Zealand pastured herds in which radiotelemetric pressure-sensitive mount detectors were used to monitor estrus characteristics, no significant variation was found in the time to onset of estrus or total mounting activity when the data were grouped into 6-hour periods. A trend toward more mounting in the afternoon was observed.[5]

Environmental Temperature

In a study of effects of environmental temperature on estrous activity, as maximum daily temperatures increased from –15° C to approximately 25° C, mounting activity (mounts per hour) also increased. When temperatures peaked higher than 30° C, beyond the comfort range for cattle, mounting was less frequent.[22] During cold weather, cows exhibit more mounting activity than cows exposed to hot weather; however, during hot

weather, estrous cows tend to exhibit more secondary signs, such as rubbing, licking, and chin resting.[23] Thus, the estrus detection error rate may increase during hot weather. Reduced estrous behavior during heat stress may be due in part to the overall reduction in physical activity. It has been shown that heat stress reduced plasma concentrations of estradiol and delayed regression of the CL by 9 days in lactating cows.[24] It was suggested that heat stress prolonged luteal phases as a result of inadequate estradiol secretion. Such changes potentially may reduce expression of estrus. Heat abatement strategies should improve estrus detection rate and conception rate.

Nutrition and Level of Milk Production

Nutrition, specifically energy balance, has an impact on initiation of postpartum estrous cycles. The interval from parturition to ovulation is inversely related to average energy balance during the initial 20 days of lactation.[25] Mounting activity has been shown to be delayed in cows that lost more weight during the dry period or postpartum period than herdmates with minimal weight loss. For cows that have initiated estrous cycles, however, the evidence is less conclusive for an adverse effect of body condition loss on intensity and duration of estrous behavior. Few data are available concerning the effects of mineral and vitamin nutrition on estrous behavior.

Although reduced estrous expression was reported in higher-producing cows, the relationship between level of milk production and intensity of estrous behavior is unclear.[26] By contrast, estrus detection rates were highest for cows producing slightly above average and did not differ between cows in the highest and lowest milk production groups.[27]

METHODS TO ENHANCE EFFICIENCY OF ESTRUS DETECTION

Management Requirements

To achieve goals of accurate and efficient estrus detection, the management team should consider implementation of the following measures.

1. Improve cow identification. Studies have shown that as many as 25% of the cattle presented for insemination are not in estrus, and poor cow identification can be one cause of this problem in large herds. Legible neck chain numbers and large ear tags with bright contrasting colors can aid in accurate identification and reduce mistakes.

2. Promote and observe cow interaction. Isolate cows suspected to be in estrus with a sexually active cow or heat detector animal. Estrus may not be observed in a large group setting, but when isolated with an estrous female or detector animal, a cow possibly in estrus may be more likely to exhibit standing behavior. Introduction of a new or novel cow has been shown to increase estrus activity.[18] Watch for sexually active groups of cattle. Cows in proestrus or estrus tend to congregate together. Moving cattle to a separate area may stimulate estrous behavior.

3. Maximize nutrition and health. Adjust feeding programs so cows calve in proper body condition, weight loss is minimized during dry period and lactation, and dry matter intake is maximized. When cows have sore feet and legs, expression of estrous behavior is less likely, and feed intake is reduced, which may lead to severe negative energy balance with delayed onset of ovarian activity. Minimize these problems by trimming hoofs on a regular basis, and treat infected feet as soon as the problem is apparent. Proper use of a footbath is helpful.

4. Provide good footing surface. Slippery and muddy conditions severely inhibit mounting activity. Provide an area with a good footing surface where cattle are free to interact, with few obstacles to hinder movement.

5. Maintain and use accurate records. All estrous periods should be recorded, including those for animals that are not to be inseminated. Estrus detection will improve if future estrous periods are anticipated. Dairy management computer programs generate action lists indicating cows to watch closely for estrus or cows not observed in estrus since calving. Breeding wheels and estrous expectancy charts also are effective.

6. Establish employee responsibility. When several people are working with a herd, one person should be assigned the responsibility for estrus detection and allowed adequate time to do the job properly. All employees, however, should be trained to recognize signs of estrus, the importance of estrus detection, and to promptly report information relative to estrous behavior to the responsible person. Training sessions to review basics of estrus detection, identify problems, set goals, and assess progress in achieving goals should be conducted periodically with the entire management team in attendance.

7. Implement aggressive detection measures. Use time efficiently. Cattle should be observed at times and in an area where they are likely to mount. Even though loose housing systems provide more time for cow interaction, cattle must be observed frequently. Pastured cattle should be moved to an area where they can easily be observed. In stanchion and tie-stall housing systems, cattle should be released twice daily for 20 to 30 minutes for estrus detection. Turn cows out when time can be devoted to observing them. Avoid scheduling observation periods at feeding time or during the warmest hours during summer.

8. Develop estrous synchronization programs. Inducing estrus and ovulation using various synchronization programs increases the probability of identifying females in estrus because (1) the management team is more committed to detecting estrus; (2) estrus is anticipated on specific days and is more likely to be observed; and (3) as illustrated in Table 36-3, an increase in the number of animals in estrus simultaneously results in more mounts per estrous cow, thus increasing the likelihood of observing estrus.

Estrus Detection Aids

In addition to the use of accurate record systems and synchronization programs, as described previously, a variety of additional aids to estrus detection are available. Tradi-

tional aids include pressure-sensitive mount detectors, tailhead markings, and detector animals. Monitoring electrical resistance of reproductive tract tissue, pedometry, and electronic pressure-sensitive mount detectors are newer concepts.

Pressure-Sensitive Mount Detectors and Tailhead Markings

Mount detectors are applied with adhesive to the topline over the rump forward toward the hooks, according to the size of the animals. Sustained pressure for several seconds by the mounting animal will activate the device. Proper placement of the device is important. Marking the tailhead with livestock chalk, paint, or crayon and observing for evidence of rubbed-off or smeared markings constitute a less expensive approach than use of pressure-sensitive mount detectors that has gained popularity in larger herds. Research has confirmed that using these conventional systems without supporting visual observation for estrus results in lower pregnancy rates. Both methods can be useful for improving the efficiency of estrus detection during a synchronization program. This system works most effectively in loose-housing operations in which cattle can be restrained in self-locking headgates to be marked and observed for evidence of smeared or rubbed-off markings, which indicate that the animal was mounted. Research at Washington State University documented the effectiveness of an aggressive prostaglandin, tailhead marking, and timed-insemination program for dairy heifers.[28] Of the heifers inseminated as indicated by rubbed-off markings, only 2.5% had high P_4 levels. The conception rate was 62%. Thus, the error rate of this breeding program was low. Success depends on frequency of observation, proper placement of devices or markings, and the density and housing arrangement of the cattle.

Estrus-Detector Animals

Use of estrus-detector animals can improve estrus detection if this approach is conducted properly and is used to supplement visual observation. On an individual basis, however, vasectomized or surgically altered bulls and hormonally treated animals vary in their sexual aggressiveness. Such animals have the potential to increase the sexual activity in the herd. The more animals sexually active at one time, the more mounting will occur for each animal in estrus. Surgically altering the penis of a bull to prevent intromission may be more costly than vasectomy, but this method is preferred because vasectomized bulls can copulate and possibly spread disease. Such bulls can be used with beef cattle to stimulate early postpartum estrous activity, termed the **male effect**, and then remain with the herd for enhanced estrus detection during an artificial insemination program. Disadvantages include the hazards of keeping a bull, cost of vasectomy or surgical alteration, perception as an animal welfare issue, and general costs of maintaining a bull.

Testosterone causes increased sexual aggressiveness when injected or implanted into steers, cows, or heifers. Nonlactating cows or heifers, even freemartin heifers, are preferred candidates for this treatment and tend to be more docile than surgically altered or vasectomized bulls. Use of androgenized heifers, implanted with testosterone propionate and estradiol benzoate and equipped with chin-ball markers, for estrus detection during 30-minute periods increased the likelihood of detection of estrus.[29] By contrast, other investigators reported significant variability among androgenized heifers and relative ineffectiveness of this method for improving the efficiency of estrus detection.[30] Of note, however, none of the cows identified with use of androgenized heifers had high milk P_4 concentrations. The combined use of mount detection devices and androgenized heifers improved estrus detection over that achieved with each system used alone.

Electrical Resistance of Reproductive Tissue Fluids

Early research performed in Europe showed that electrical resistance (ER) of vaginal fluids decreased during proestrus and estrus. Other studies have validated this concept. Probes that measure ER of vaginal fluids are commercially available. The challenge is to adapt this technology to the herd management situation. ER measurements vary among cows; however, monitoring relative changes within cows during the estrous cycle can provide useful information. Once ER readings begin to decline, the cow should be probed frequently until the lowest reading is obtained. Theoretically, this lowest reading coincides with the time of estrus. In several studies summarized by Lehrer and co-workers, ER measurements have been useful to time insemination and improve conception rates, with detection efficiency ranging from 65% to 82% and accuracy between 57% and 82%.[31] This tool is expensive and labor intensive, because cattle must be probed frequently to detect significant changes in ER. The probe should be disinfected and dried each time it is used.

Miniature radiotelemetric sensors have been implanted experimentally within vulvar tissue to continuously monitor ER.[32] A strong relationship was found between concentrations of ovarian steroids and ER. These findings support this concept as a valid aid to estrus detection.

Pedometry

It has been well documented that cattle are more active during estrus and spend more time walking and standing than resting. Various pedometers have been developed. Earlier prototypes generally were unreliable because of a high rate of false positives and required frequent replacement. As pointed out by Lehrer and co-workers in a review of the technology, the efficiency of pedometry has been variable among studies and ranges from 60% to 100%, whereas the accuracy of detection of estrus ranged from 22% to 100%.[31]

Electronic pedometer systems have been installed in commercial herds. In a recent review, several concepts related to pedometry were noted.[33] Activity information usually is obtained by an interrogation unit in the milking parlor and transmitted to a herd management program for analysis. Devices are mounted on the neck or leg of the cow. Activity is measured by a mercury switch, which is activated by movement of the cow. The most common method for determination of the onset of estrus is attainment of a certain threshold of relative

activity compared with earlier measurements. Using a pedometer system that monitored steps per hour, Arney and co-workers showed a linear increase in activity during the 72 to 16 hours before estrus and a rapid increase between 16 hours and estrus. Following estrus, activity decreased exponentially.[34] Using pedometers attached to the inside of the cow's right hind leg, Maajtje and co-workers calculated the optimal time for insemination to be 11.8 hours after increased activity.[35] To be most effective in determining proper timing of insemination, pedometry systems should incorporate real-time data acquisition.[21]

Electronic Pressure-Sensitive Mount Detectors

A pressure-sensing radiotelemetric estrus detection system that monitors frequency and duration of mounting is commercially available to dairy and beef producers. This externally mounted device, which captures mounting information and transmits to a receiver interfaced with a computer, has been tested.[5,6,36,37] Results showed the accuracy of estrus detection for this system to be similar to that for visual observation for estrus, with rates of 96% and 94%, respectively. The efficiency of detection increased from 51% for visual observation to 91% with this system, however.[36] On the basis of timing of ovulation as determined by ultrasonography, the computer information should be accessed at least twice daily so inseminations can be properly timed. As noted in a review of this technology, duration of estrus varied among cows in the same herd and among studies using this technology.[21]

An alternative approach that has been tested but not commercially available involves a pressure-sensitive electronic device implanted subcutaneously in the cow's midsacral region that monitors duration and frequency of mounts received.[4] In addition to monitoring mounting, this device also has the capability to respond to skin movement across the midsacral region of the topline as the cow walks.[38] Combining the pedometry component with the mount monitor would make this device a very effective detection aid.

As shown in previous studies, the use of several detection aids or monitoring two or more aspects of estrous behavior is superior to a single method for improving the efficiency of estrus detection. The cost, durability, accuracy, functional life, maintenance requirements, access to and interpretation of data, length of time these devices remain attached to the animal, and labor commitment are factors that must be considered in evaluating any new estrus detection technology. As noted in a review of estrus detection technology,[4] unbiased field-based research data are necessary to document the effectiveness of such technology.

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CHAPTER 37

Progestogen-Based Estrus Synchronization for Beef Replacement Heifers and Cows

DAVID J. PATTERSON and MICHAEL F. SMITH

The beef cattle industry has seen rapid gains in economically desirable traits, largely as a result of the selection and expanded use of genetically superior sires made available through artificial insemination (AI). Recent surveys indicate, however, that less than 5% of the beef cows in the United States are bred by AI, and only half of the cattle producers who practice AI use any form of estrus synchronization to facilitate their AI programs. The inability to predict time of estrus for individual cows or heifers in a group often makes it impractical to use AI because of the labor required for detection of estrus. Available procedures to control the estrous cycle of the cow can improve reproductive rates and facilitate genetic progress. These procedures include synchronization of estrus in cycling females and induction of estrus accompanied by ovulation in heifers that have not yet reached puberty or among cows that have not returned to estrus after calving.

The following protocols and terms are referred to throughout this chapter.

Protocols

$PGF_{2\alpha}$: Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$): (Lutalyse, Estrumate, ProstaMate, InSynch).

MGA-$PGF_{2\alpha}$: Melengestrol acetate (MGA) (0.5 mg/day per animal) is fed for a period of 14 days, with

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MGA-$PGF_{2\alpha}$: Melengestrol acetate (MGA) (0.5 mg/day per animal) is fed for a period of 14 days, with

$PGF_{2\alpha}$ administered 17 to 19 days after MGA withdrawal.

GnRH-$PGF_{2\alpha}$ (Select Synch): Gonadotropin-releasing hormone (GnRH) injection (Cystorelin, Factrel, Fertagyl), followed in 7 days with an injection of $PGF_{2\alpha}$.

MGA-GnRH-$PGF_{2\alpha}$ (MGA Select): MGA is fed for 14 days, GnRH is administered 10 or 12 days after MGA withdrawal, and $PGF_{2\alpha}$ is administered 7 days after GnRH.

7-11 Synch: MGA is fed for 7 days, $PGF_{2\alpha}$ is administered on the last day MGA is fed, GnRH is administered 4 days after the cessation of MGA, and a second injection of $PGF_{2\alpha}$ is administered 11 days after MGA withdrawal.

Terms

Estrus response: The number of females that exhibit estrous behavior during a synchronized period.

Synchronized period: The period of time during which estrous behavior is expressed after treatment.

Synchronized conception rate: The proportion of females that become pregnant of those exhibiting estrus and inseminated during the synchronized period.

Synchronized pregnancy rate: The proportion of females that become pregnant from inseminations during the synchronization period of the total number treated.

A successful estrus synchronization program brings a number of well-recognized advantages to the beef cattle operation: (1) Cows or heifers are in estrus during a predictable interval, which allows for the use of AI, embryo transfer, or other planned reproductive techniques; (2) the time required to detect estrus is reduced, which in turn decreases labor expense associated with the breeding program; (3) cattle will conceive earlier during the breeding period; and (4) calves will be older and weigh more at weaning.

To maximize the benefits from estrus synchronization, females should be selected for a program when the following conditions are met[1]:

- Adequate time has elapsed between calving and the time synchronization treatments are implemented (a minimum of 40 days post partum at the beginning of treatment is suggested).
- Cows are in average or above-average body condition (scores of at least 5 on a scale of 1 to 9 on which 1 = emaciated and 9 = obese).
- Cows experience minimal calving problems.
- Replacement heifers are developed to prebreeding target weights of at least 65% of projected mature weight.
- Reproductive tract scores (RTSs) are assigned to heifers no more than 2 weeks before initiation of treatment (scores of 3 or higher on a scale of 1 to 5), and at least 50% of the heifers are assigned an RTS of 4 or 5.

DEVELOPMENT OF METHODS TO SYNCHRONIZE ESTRUS

The development of methods to control the estrous cycle of the cow has occurred in five distinct phases. The physiologic basis for estrus synchronization followed the discovery that progesterone inhibited preovulatory follicular maturation and ovulation. Regulation of estrous cycles was believed to be associated with control of the corpus luteum, whose lifespan and secretory activity are regulated by trophic and lytic mechanisms. **Phase I** included efforts to prolong the luteal phase of the estrous cycle or to establish an artificial luteal phase by administering exogenous progesterone. Later, progestational agents were combined with estrogens or gonadotropins in **phase II**, whereas **phase III** involved $PGF_{2\alpha}$ and its analogues as luteolytic agents. Treatments that combined progestational agents with $PGF_{2\alpha}$ characterized **phase IV.**

Precise monitoring of ovarian follicles and corpora lutea over time by transrectal ultrasonography expanded our understanding of the bovine estrous cycle and particularly the change that occurs during a follicular wave. Growth of follicles in cattle occurs in distinct wavelike patterns, with new follicular waves occurring approximately every 10 days (6- to 15-day range). We now know **(phase V)** that precise control of estrus and ovulation requires the manipulation of both follicular waves and luteal lifespan.

A single injection of GnRH to cows at random stages of their estrous cycles causes release of luteinizing hormone leading to synchronized ovulation or luteinization of most large dominant follicles. Consequently, a new follicular wave is initiated in all cows within 2 to 3 days after GnRH administration. Luteal tissue that forms after GnRH administration is capable of undergoing $PGF_{2\alpha}$-induced luteolysis 6 or 7 days later.[2] This method is referred to as the GnRH-$PGF_{2\alpha}$ protocol throughout this chapter. The GnRH-$PGF_{2\alpha}$ protocol increased estrus synchronization rate in both beef[3,4] and dairy[5] cattle. A drawback of this method is that approximately 5% to 15% of the cows are detected in estrus on or before the day of $PGF_{2\alpha}$ injection, so that a smaller proportion of females are detected in estrus and inseminated during the synchronized period.[6]

THE MELENGESTROL ACETATE PROGRAM

This section reviews recently developed methods using MGA to control estrous cycles of cows or heifers in breeding programs involving natural service or artificial insemination. Four methods are outlined for using MGA (MGA Premix, Pfizer Animal Health, New York, NY) to facilitate estrus synchronization in heifers or cows. The choice of which system to use depends largely on a producer's goals. MGA is the common denominator in each of the systems described. MGA is an orally active progestogen that will suppress estrus and prevent ovulation when consumed by cows or heifers on a daily basis.

MGA may be fed with a grain or a protein carrier and either top-dressed onto other feed or batch-mixed with larger quantities of feed. MGA is fed at a rate of 0.5 mg/animal/day in one feeding. The duration of

feeding may vary between protocols, but the level of feeding is consistent and critical to success. Animals that fail to consume the required amount of MGA on a daily basis may prematurely return to estrus during the feeding period. This can be expected to reduce the synchronization response. Therefore, adequate bunk space must be available so that all animals consume feed simultaneously.

Animals should be observed for behavioral signs of estrus each day of the feeding period. This may be done as animals approach the feeding area and before feed distribution. This practice will ensure that all females receive adequate intake. Cows and heifers will exhibit estrus beginning 48 hours after MGA withdrawal, and this behavior will continue for 6 to 7 days. It generally is recommended that females exhibiting estrus during this period not be inseminated or exposed to natural service because of the reduced fertility among such animals at the first heat after MGA withdrawal.

Method 1: MGA with Natural Service

The simplest MGA-based method of estrus synchronization involves using bulls to breed synchronized groups of females. This practice is especially useful in helping producers make a transition from natural service to AI. In this process, cows or heifers receive the normal 14-day feeding period of MGA and are then exposed to fertile bulls about 10 days after MGA withdrawal (Fig. 37-1).

This system works effectively; however, careful attention to bull-to-female ratios is indicated. A ratio of 15 to 20 synchronized females per bull is recommended. Age and breeding condition of the bull and results of breeding soundness examinations should be considered carefully.

Method 2: MGA plus Prostaglandin

A more precise means of estrous cycle control involves the combination of MGA with $PGF_{2\alpha}$. $PGF_{2\alpha}$ is a luteolytic compound normally secreted by the uterus of the cow. $PGF_{2\alpha}$ can induce luteal regression but cannot inhibit ovulation. When $PGF_{2\alpha}$ is administered in the presence of a functional corpus luteum (CL) during days 6 to 16 of the estrous cycle, premature regression of the CL begins and the cow returns to estrus.

In this program, $PGF_{2\alpha}$ should be administered 19 days after the last day of MGA feeding. This treatment places all animals in the late luteal stage of the estrous cycle at the time of injection, which shortens the synchronized period and maximizes conception rate (Fig. 37-2). Although a 19-day interval is optimal, 17- to 19-day intervals produce acceptable results and provide flexibility for extenuating circumstances.[7,8] Any of the four available $PGF_{2\alpha}$ products can be used for synchronization of estrus in cattle after the MGA treatment. Label-approved dosages differ with each of these products; the practitioner should carefully read and follow directions for proper administration before their use.

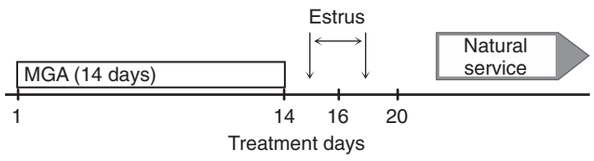

Fig. 37-1 Melengestrol acetate (MGA) and natural service. (Adapted from Patterson et al.[9])

Figure 37-3 illustrates the distribution of estrus comparing the MGA-$PGF_{2\alpha}$ system and an MGA-only system.[9] The combined MGA-$PGF_{2\alpha}$ system is best suited for use with AI programs because of the high degree of synchrony that can be achieved, which decreases the amount of time required for detection of estrus. Under natural mating conditions, distributing estrus over several additional days may be advantageous, to prevent overworking of bulls used in these programs.

Table 37-1 provides a summary of field trials involving heifers in which MGA was used in conjunction with natural service or MGA-$PGF_{2\alpha}$ was used before AI.[9] One of the major advantages in using MGA to control estrous cycles of cattle, as seen from the data presented in the table, is the flexibility in matching specific synchronization protocols with the particular management system involved.

Method 3: MGA Select

The MGA Select program[10] (Fig. 37-4) is useful in maximizing estrus response and reproductive performance in postpartum beef cows. The MGA Select protocol is a simple program that involves feeding MGA for 14 days, followed by an injection of GnRH on day 26 and an injection of $PGF_{2\alpha}$ on day 33. The addition of GnRH to the 14- to 19-day MGA-$PGF_{2\alpha}$ protocol improves synchrony of estrus while maintaining high fertility in postpartum beef cows.

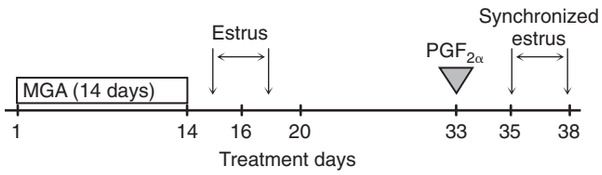

Fig. 37-2 The melengestrol acetate–prostaglandin $F_{2\alpha}$ (MGA-$PGF_{2\alpha}$) protocol. (Adapted from Brown et al[7] and Lamb et al.[14])

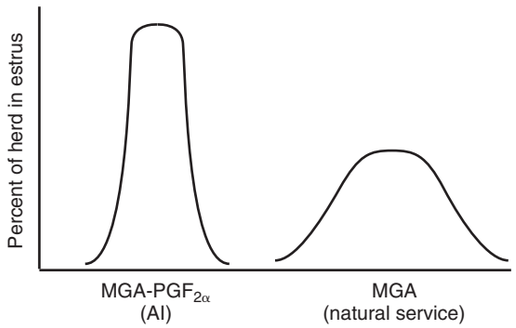

Fig. 37-3 Distribution of estrus comparing the melengestrol acetate–prostaglandin $F_{2\alpha}$ (MGA-$PGF_{2\alpha}$) system with an MGA-only system. (Adapted from Patterson et al.[9])

Table **37-1**

MGA versus MGA-$PGF_{2\alpha}$ for Estrus Synchronization: Summary of Field Trials*

Breeding Program	No. of Heifers	Estrus Response Rate	Synchronized Conception Rate	Synchronized Pregnancy Rate
Natural service (with MGA)	1749	—	—	1151/1749 (66%)
Artificial insemination (with MGA-$PGF_{2\alpha}$)	4245	3354/4245 (79%)	2414/3354 (72%)	2414/4245 (57%)

*MGA was given before natural service; MGA-$PGF_{2\alpha}$ was given before artificial insemination.
MGA, melengestrol acetate; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.
Data from Patterson DJ, Wood SL, Kojima FN, Smith MF: Current and emerging methods to synchronize estrus with melengestrol acetate. Proceedings of the 49th Annual Beef Cattle Short Course, "Biotechnologies of Reproductive Biology." University of Florida, Gainesville, FL, 2000, pp 45–66.

Table **37-2**

MGA Protocols and Cyclicity Status, with Clinical Data by Age

Treatment	Age Group (yr)	No. of Cows	Days Post Partum*	Body Condition Score†	% Cycling‡
MGA-$PGF_{2\alpha}$	2, 3, 4	52	47	5.2	35
	5+	48	39	5.2	15
	Total	100	44	5.2	25
MGA-GnRH-$PGF_{2\alpha}$	2, 3, 4	53	47	5.3	38
	5+	48	40	5.3	13
	Total	101	44	5.3	26

*Average number of days post partum on the day treatment with MGA began.
†Body condition scores were assigned 1 day before treatment with MGA was initiated using a scale 1 = emaciated to 9 = obese.
‡Cyclicity was determined from two blood samples for progesterone obtained 10 days and again 1 day before initiation of treatment with MGA.
GnRH, gonadotropin-releasing hormone; MGA, melengestrol acetate; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.
Data from Patterson DJ et al. (unpublished).

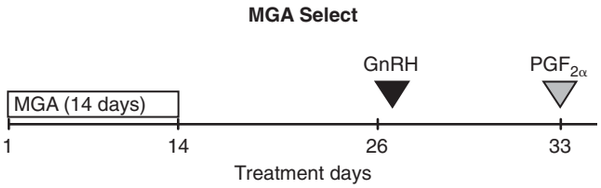

Fig. 37-4 The MGA Select protocol. Melengestrol acetate (MGA) is fed for a period of 14 days, followed in 12 days (day 26) by an injection of gonadotropin-releasing hormone (GnRH) and then prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 19 days after MGA withdrawal (day 33).

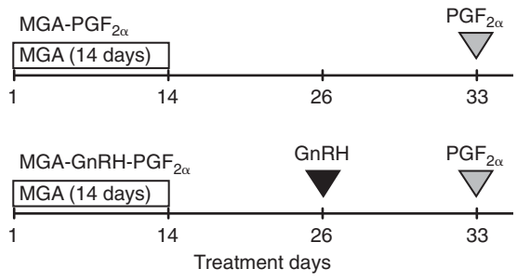

Fig. 37-5 Cows were fed melengestrol acetate (MGA) for 14 days; 19 days after MGA withdrawal, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was administered to all cows. Gonadotropin-releasing hormone (GnRH) was administered to one half of the cows 7 days before $PGF_{2\alpha}$. (Data from Patterson DJ et al.[11])

We conducted experiments during the spring 2000 and 2001 breeding seasons to compare the 14- to 19-day MGA-$PGF_{2\alpha}$ protocol with or without the addition of GnRH on day 12 after MGA withdrawal and 7 days before $PGF_{2\alpha}$ in postpartum-suckled beef cows[11] (Fig. 37-5). These experiments were conducted at the University of Missouri's Thompson Farm at Spickard, Missouri.

The results from the study conducted during the 2001 breeding season are summarized in several tables. Table 37-2 presents the number of cows within age group by treatment, the average number of days post partum and body condition score on the first day of MGA feeding, and the percentage of cows that were cycling before the treatment with MGA began. Cyclicity status was based on progesterone levels in two blood samples obtained 10 days before and on the first day of MGA feeding.

Table 37-3 provides a summary of estrus response, synchronized conception and pregnancy, and final pregnancy rates for cows assigned to the two treatments. Estrus response was significantly higher among MGA Select–treated cows than among the MGA-$PGF_{2\alpha}$–treated cows. Synchronized pregnancy rates were higher among cows 5 years of age and older assigned to MGA Select treatment.

Table **37-3**

MGA Protocols and Estrus, Conception, and Pregnancy Rates

Treatment	Age Group (yr)	Estrus Response Rate	Synchronized Conception Rate	Synchronized Pregnancy Rate	Final Pregnancy Rate
MGA-$PGF_{2\alpha}$	2, 3, 4	44/52 (85%)	36/44 (82%)	36/52 (69%)	49/52 (94%)
	5+	32/48 (67%)	22/32 (69%)	22/48 (46%)[a]	48/48 (100%)
	Total	76/100 (76%)[a]	58/76 (76%)	58/100 (58%)	97/100 (97%)
MGA-GnRH-$PGF_{2\alpha}$	2, 3, 4	46/53 (87%)	33/46 (72%)	33/53 (62%)	51/53 (96%)
	5+	42/48 (88%)	34/42 (81%)	34/48 (71%)[b]	47/48 (98%)
	Total	88/101 (87%)[b]	67/88 (76%)	67/101 (66%)	98/101 (97%)

[a,b]Percentages within column and category with unlike superscripts are different ($P < 0.05$).
GnRH, gonadotropin-releasing hormone; MGA, melengestrol acetate; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.
Data from Patterson DJ, Graham KK, Kerley MS, et al: Estrus synchronization in postpartum suckled beef cows using a 14-19 day melengestrol acetate (MGA)–prostaglandin $F_{2\alpha}$ (PG) protocol with or without the addition of GnRH. *J Anim Sci* 2001;79:250 Suppl 1.

Table **37-4**

Fixed-Time Artificial Insemination and Final Pregnancy Rates in Two Herds of MGA-Treated and Control Cows

	PREGNANCY RATE		
Category	Herd 1	Herd 2	Combined
Fixed-time artificial insemination			
MGA-treated	26/45 (58%)	44/70 (63%)[a]	70/115 (61%)[a]
Control	23/45 (51%)	30/67 (45%)[b]	53/112 (47%)[b]
End of breeding season (final rates)			
MGA-treated	38/45 (84%)	64/70 (91%)	102/115 (89%)
Control	38/45 (84%)	59/67 (88%)	97/112 (87%)

[a,b]Percentages within column and category with unlike superscripts are different ($P < 0.05$).
Data from Perry GA, Bader JF, Smith MF, Patterson DJ: Evaluation of a fixed-time artificial insemination protocol for beef cows. *J Anim Sci* 2001;79:462 Suppl 1.

The objective of a second experiment during the spring 2000 breeding season was to determine if MGA pretreatment could improve conception rates with a GnRH-$PGF_{2\alpha}$-GnRH protocol.[12] Cows from two University of Missouri herds—Greenley Farm ($N = 90$) (herd 1) and South Farm ($N = 137$–) (herd 2) were assigned by age and days post partum to one of two treatments. Control and MGA-treated (Fig. 37-6) cows were fed a supplement carrier with or without MGA for 14 days. GnRH was administered to all cows 12 days after MGA or carrier withdrawal and 7 days before $PGF_{2\alpha}$. All animals were administered GnRH and artificially inseminated 72 hours after $PGF_{2\alpha}$.

Pregnancy rates to fixed-time AI were determined 50 days after insemination (Table 37-4). No difference was observed between treatments in herd 1 (MGA = 58% [26/45]; control = 51% [23/45]). A difference ($P < 0.03$) in pregnancy rate to fixed-time AI between treatments, however, was found for herd 2 (MGA = 63% [44/70]; control = 45% [30/67]). Furthermore, when the data from

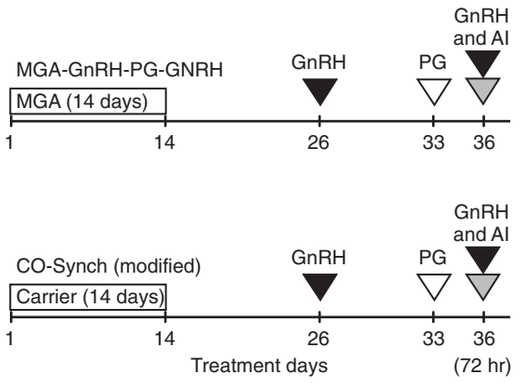

Fig. 37-6 Control and MGA-treated cows were fed a supplement carrier with or without MGA for 14 days. GnRH was administered to all cows 12 days after MGA or carrier withdrawal and 7 days before $PGF_{2\alpha}$.

both locations were combined, the overall difference remained significant (MGA = 70/115 [61%]; control = 53/112 [47%]; $P < 0.05$). These findings indicate that pregnancy rates resulting from fixed-time insemination are improved significantly when treatment with MGA precedes the GnRH-$PGF_{2\alpha}$-GnRH protocol.

Method 4: 7-11 Synch

Recently we developed an estrus synchronization protocol for beef cattle that was designed to (1) shorten the feeding period of MGA without compromising fertility and (2) improve synchrony of estrus by synchronizing development and ovulation of follicles from the first wave of development[6] (Fig. 37-7A). This new treatment, 7-11 Synch, was compared with the GnRH-$PGF_{2\alpha}$ protocol. Synchrony of estrus during the 24-hour peak response period (42 to 66 hours) was significantly higher among 7-11 Synch–treated cows. Furthermore, the distribution of estrus was reduced from 144 hours for GnRH-$PGF_{2\alpha}$–treated cows to 60 hours for cows assigned to the 7-11 Synch treatment[6] (Fig. 37-7B) The 7-11 Synch protocol resulted in a higher degree of estrus synchrony (91%) and greater AI pregnancy rate (68%) during a 24-hour peak response period compared with the GnRH-$PGF_{2\alpha}$ protocol (69% and 47%, respectively).

Additional Considerations

An additional consideration for methods 2, 3, and 4 pertains to cows or heifers that fail to exhibit estrus after the last $PGF_{2\alpha}$ injection. Such cows or heifers can be reinjected with $PGF_{2\alpha}$ 11 to 14 days after the last injection of $PGF_{2\alpha}$ was administered. These females then are observed for signs of behavioral estrus for an additional 6 to 7 days. This procedure maximizes the number of females inseminated within the first 2 weeks of the breeding period. Cows that were inseminated during the first synchronized period should not be reinjected with $PGF_{2\alpha}$. In addition, the decision to use method 3 or 4 in heifers should be based on careful consideration of the heifer's age, weight, and pubertal status.

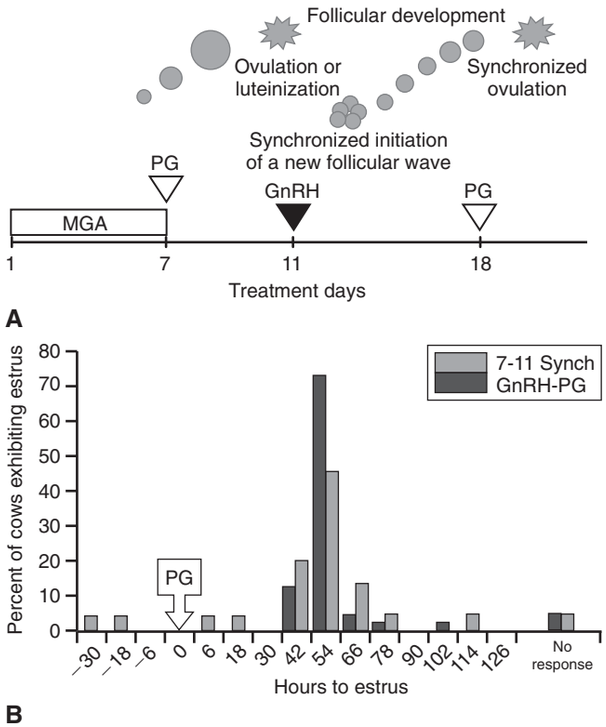

Fig. 37-7 **A,** Treatment schedule and events associated with the 7-11 Synch protocol. **B,** Estrus response of cows treated with the 7-11 Synch or GnRH-$PGF_{2\alpha}$ protocols. (Data from Kojima et al.[6])

INTRAVAGINAL PROGESTERONE-RELEASING INSERT

The presence of anestrous beef heifers and cows at the start of the breeding season is a major limitation to the success of a $PGF_{2\alpha}$-based estrus synchronization program. Administration of a progestogen improves estrus synchronization results through the induction of estrus and ovulation, as well as by providing time for corpora lutea to become responsive to the luteolytic action of $PGF_{2\alpha}$ (>5 days after estrus; day 0 = estrus). A controlled internal drug release device (CIDR) (EAZI-BREED, Pfizer Animal Health, New York, NY) has been developed for the intravaginal release of progesterone and has been proved to be effective for inducing and synchronizing estrus in heifers and cows.[13] CIDRs are marketed in more than 30 countries, including the United States.

Two types of CIDR inserts have been used worldwide: CIDR 1380, which contains 1.38 g of progesterone, and CIDR B, which contains 1.9 g of progesterone. In the absence of a corpus luteum, a CIDR functions as an artificial corpus luteum and suppresses estrus and ovulation for 7 days or longer. CIDR inserts consist of a T-shaped nylon backbone that is coated with a silicone layer containing 10% progesterone by weight. The CIDR inserts are placed into the vagina with a lubricated applicator, after disinfection of the vulva. The device has a flexible polyester tail that protrudes from the vulva and is easily removed by pulling the polyester tail. Although mild vaginitis is common with use of CIDR inserts, fertility is not compromised. The retention rate for CIDR inserts is approximately 95%. If the retention rate is considerably less than 95%, the devices may have been inserted incorrectly, or other animals may be pulling out the CIDR insert by biting on the polyester tails. In the latter case, the problem can be remedied by trimming the polyester tails. The CIDR treatment that has been approved in the United States includes insertion of a CIDR device on day 0, injection of $PGF_{2\alpha}$ on day 6, and CIDR insert removal on day 7 (Fig. 37-8, *A*). Cows and heifers typically are observed in estrus 24 to 72 hours after CIDR insert removal, and they commonly are inseminated after detection of estrus.

The efficacy of the CIDR treatment (CIDR plus $PGF_{2\alpha}$) in beef heifers and cows was evaluated in a multistate trial in Florida, Illinois, Missouri, Montana, and Nebraska).[13] This trial consisted of the following treatments: control (no treatment), $PGF_{2\alpha}$ (single injection), and CIDR plus $PGF_{2\alpha}$ (1.38 g CIDR for 7 days with $PGF_{2\alpha}$ on day 6). The proportion of heifers and cows that were detected in estrus and pregnant during the first three days of the

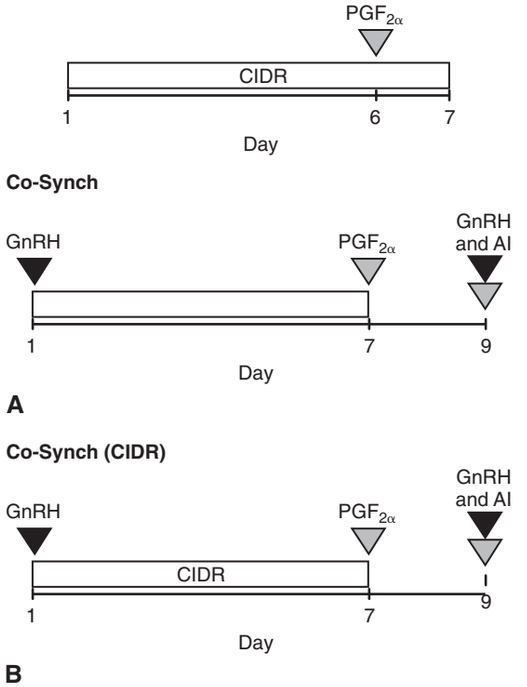

Fig. 37-8 Protocols for synchronization of estrus in beef heifers (**A**) and postpartum beef cows (**A** and **B**). "Day" refers to day of treatment. *Protocols:* CIDR = controlled internal drug release device (an intravaginal progesterone-releasing device); $PGF_{2\alpha}$ = a single injection of prostaglandin $F_{2\alpha}$ on day 6 or 7 to induce regression of luteal tissue; GnRH = a single injection of gonadotropin-releasing hormone to induce ovulation and formation of luteal tissue; AI = artificial insemination on day 9 of treatment for the CO-Synch protocol (**B**) or approximately 12 hours after detection of estrus in the other protocol (**A**).

breeding season was increased for the CIDR plus $PGF_{2\alpha}$ treatment, compared with the $PGF_{2\alpha}$ and control groups. The CIDR plus $PGF_{2\alpha}$ treatment was effective in both acyclic and cyclic cattle at multiple locations.

Expanded use of AI for beef heifers and cows probably will require the ability to inseminate cows at a fixed time, resulting in pregnancy rates that exceed 50%. Efforts to develop a more effective timed insemination protocol in beef cows have recently focused on synchronizing follicular waves by injecting GnRH, followed 7 days later with an injection of $PGF_{2\alpha}$ and then a second GnRH injection plus insemination 48 hours after the $PGF_{2\alpha}$ injection (CO-Synch protocol; see Fig. 37-8, *B*). This protocol permits the synchronization of ovulation in cows but at present has not proved to be as effective in heifers. In cows that have a dominant (≥10 mm) follicle at random stages of their estrous cycle, an injection of GnRH will induce ovulation and subsequent formation of luteal tissue. A new follicular wave is initiated in heifers or cows within 2 to 3 days of GnRH-induced ovulation of a dominant follicle, synchronizing the development of a new dominant follicle. Luteal tissue that forms after GnRH administration is capable of undergoing $PGF_{2\alpha}$-induced regression 6 or 7 days following the first GnRH injection. A limitation to GnRH-$PGF_{2\alpha}$ protocols (including CO-Synch) is that approximately 5% to 15% of the cows are detected in estrus on or before the day of $PGF_{2\alpha}$ injection, so a smaller proportion of females will conceive to the timed insemination 48 hours later. To address this problem, a multistate study was conducted in which a CIDR was inserted at the time of the first GnRH injection and removed at the time of $PGF_{2\alpha}$ injection[14] (see Fig. 37-8, *B*). The rationale for CIDR treatment was to prevent cows from showing estrus before the $PGF_{2\alpha}$ injection and to provide progesterone exposure to anestrous animals in which the first GnRH injection did not induce luteal tissue formation. The study consisted of 560 suckled beef cows, and the proportion of cycling cows before treatment ranged from 72% to 83%. The pregnancy rates after timed insemination with the CO-Synch protocol were greater for the CIDR-treated cows (58%) than for the control cows (48%).

SUMMARY AND CONCLUSIONS

Expanded use of AI and/or adoption of emerging reproductive technologies for beef cows and heifers requires precise methods of estrous cycle control. Effective control of the estrous cycle requires the synchronization of both luteal and follicular functions. Efforts to develop a more effective estrus synchronization protocol have focused recently on synchronizing follicular waves by injecting GnRH followed 7 days later by injection of $PGF_{2\alpha}$ (Ovsynch, CO-Synch, Select Synch). A factor contributing to reduced synchronized pregnancy rates in dairy and beef cows managed with the preceding protocols is that 5% to 15% of cycling cows show estrus on or before $PGF_{2\alpha}$ injection. We developed new protocols for inducing and synchronizing a fertile estrus in postpartum beef cows and beef heifers in which the GnRH-$PGF_{2\alpha}$ protocol is preceded by either short- or long-term progestogen treatment.

Although other types of progestogen treatments (CIDR, PRID, and norgestomet) can be substituted in these estrus synchronization protocols, we chose to use MGA for the following reasons:

- MGA is economical to use (approximately 2 cents per animal per day).
- MGA has been cleared for use in reproductive classes of beef and dairy cattle.[15]
- Methodology and understanding of the use of MGA are documented in the literature,[16–18] dating back as early as the 1960s.
- MGA is easily administered in feed and does not require that animals be handled or restrained during administration.

Perhaps more important, at the time these programs were developed, MGA was the only progestogen approved for use and available in the United States, making research of methods to improve and broaden the scope of its application all the more significant.

Table 37-5 provides a summary of results with various estrus synchronization protocols for use in postpartum beef cows. Included are data from our own published work, in addition to unpublished data from DeJarnette and Wallace. These results provide evidence to support

Table **37-5**

Estrus Response and Fertility in Postpartum Beef Cows with Various Estrus Synchronization Protocols

Protocol	Estrus Response Rate	Synchronized Pregnancy Rate
Two-shot $PGF_{2\alpha}$	241/422 (57%)	147/422 (35%)
Select Synch	353/528 (67%)	237/528 (45%)
MGA-$PGF_{2\alpha}$ for 14–17 d	305/408 (75%)	220/408 (54%)
MGA–two shot $PGF_{2\alpha}$	327/348 (93%)	243/348 (70%)
MGA-$PGF_{2\alpha}$ for 14–19 d	161/206 (83%)	130/206 (63%)
MGA Select	174/204 (85%)	134/204 (66%)
MGA Select + GnRH at AI 7-11 Synch	Fixed-time AI 40/44 (91%)	70/115 (61%) 30/44 (68%)

AI, artificial insemination; GnRH, gonadotropin-releasing hormone; MGA, melengestrol acetate; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

our recommended sequential approach to estrus synchronization in postpartum beef cows.

Our preliminary studies identified significant improvements in specific reproductive endpoints among cows that received MGA before the administration of $PGF_{2\alpha}$, compared with cows that received $PGF_{2\alpha}$ only, including increased estrus response and improved synchronized conception and pregnancy rates. More recently we observed a significant improvement in synchrony of estrus without compromising fertility in postpartum beef cows and beef heifers that were pretreated, either short or long term, with MGA before GnRH and $PGF_{2\alpha}$ administration. We propose the general hypothesis that progestogen (MGA) treatment before the GnRH-$PGF_{2\alpha}$ estrus synchronization protocol will (1) successfully induce ovulation in anestrous postpartum beef cows and peripubertal beef heifers; (2) reduce the incidence of a short luteal phase among anestrous cows induced to ovulate; (3) increase estrus response, synchronized conception, and pregnancy rate; and (4) increase the likelihood of successful fixed-time insemination. The cumulative data suggest that new methods of inducing and synchronizing estrus for postpartum beef cows and replacement beef heifers in which a progestogen is included in the GnRH-$PGF_{2\alpha}$ protocol offer significantly greater potential to more effectively synchronize estrus, with resulting high fertility.

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CHAPTER 38

Ovulation Synchronization Strategies in Dairy Cattle Using $PGF_{2\alpha}$ and GnRH

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Synchronization of ovulation using timed injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and gonadotropin-releasing hormone (GnRH)—the Ovsynch program of ovulation management—improves pregnancy rates and reduces days open in lactating dairy cows[1,2] (Fig. 38-1). Inadequate pregnancy rates are one of the primary obstacles to dairy farm profitability and sustainability.[3] During the past 50 years, pregnancy rates of lactating dairy cows progressively decreased, primarily because of low and steadily declining conception rates (CRs) and estrus detection rates (EDRs).[4,5] CR in cows decreased from approximately 70%[6,7] to 35%,[8,9] although CR in heifers remained steady at approximately 70%.[2,7] Current reports indicate that EDR is approximately 32% in lactating cows[4] and 70% in heifers. Poor EDR in cows may be attributed to changes in hormonal concentrations between the two groups,[10,11] as well as differences in environment—lactating dairy cows spend more time on surfaces with poor footing[12,13] and are more susceptible to heat stress.[14] Currently, the effectiveness of Ovsynch in lactating dairy cows is attributable primarily to an increase in EDR, or artificial insemination (AI) service rate, because all cows, regardless of cycling status or stage of estrous cycle, can be inseminated by appointment[1,2] without the need for detection of estrus. Thus, Ovsynch can be effectively used to control time to first and subsequent inseminations by AI, thereby maximizing service rate and improving overall EDR.[1]

Ovsynch is the basis for most timed-AI programs in dairy cows in the United States. Ovsynch was developed in the early 1990s at the University of Wisconsin–Madison by Pursley and Wiltbank.[15] The objectives of the original Ovsynch research were to (1) hormonally control the onset of a new follicular wave, (2) control the lifespan of the spontaneous and induced CL, and (3) control the time of ovulation of the dominant follicle (DF).

Three hormonal injections were needed to accomplish these three objectives (see Fig. 38-1). The first GnRH injection caused ovulation, or luteinization, if a functional DF was present in the ovary. Subsequently, if ovulation occurred, a new follicular wave emerged approximately 1.5 to 2 days later.[15] GnRH did not cause ovulation if the stage of follicle development was in the first 3 days of a spontaneous follicular wave. The newly induced wave, or a spontaneous wave if ovulation did not occur, was allowed to develop, with selection and dominance of a DF during the following 7 days. At that time, $PGF_{2\alpha}$ was administered to induce luteolysis, thus allowing for further growth and maturation of the DF. Then, 48 hours later, a second GnRH injection induced a preovulatory luteinizing hormone (LH) surge that triggered ovulation approximately 28 hours after treatment with GnRH. Cows treated with Ovsynch demonstrated overall CRs similar to those obtained in cows that had been bred to detected estrus (37% versus 39%, respectively; $P > 0.05$).[2]

This chapter discusses how Ovsynch works and how it may be altered in the future to improve the percentage of cows synchronized, and the possibility of improving

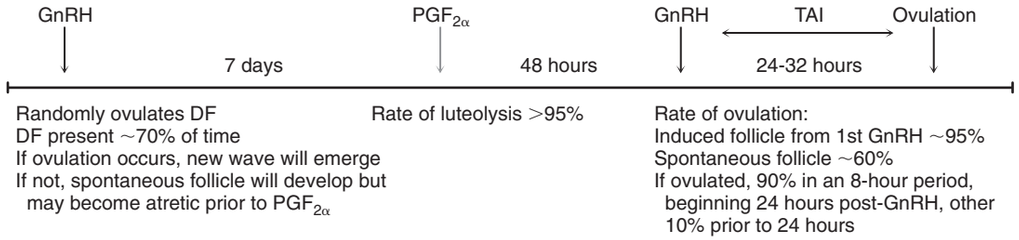

Fig. 38-1 Description of the original Ovsynch program, with outcomes from each hormonal injection.

CR by means of greater control over the ovulatory follicle.

SYNCHRONIZATION RATES WITH OVSYNCH

The main limitation of Ovsynch is the wide variability in **synchronization rate** (SR), defined in this protocol as regression of a CL and induction of ovulation in response to the final GnRH. Approximately 10% to 30% of cows may not synchronize.[9,16–18] It is likely that nonsynchronized cows could receive AI at an inappropriate time relative to ovulation, thereby decreasing their chances of becoming pregnant. Nevertheless, with SRs in this range, it is impressive that CR after Ovsynch is similar to that with AI at the time of detected estrus.

Vasconcelos and associates attributed most of the variation in SRs to the stage of the estrous cycle at which Ovsynch was initiated.[18] Cows started on Ovsynch at mid-cycle (days 5 to 9 of the cycle) had a greater probability of synchronizing and therefore had a greater chance of conception. The key reasons for increased SR in mid-cycle were the presence of a DF responsive to GnRH during the first follicular wave and a CL that remained functional during the 7-day period between GnRH and $PGF_{2\alpha}$. Early in the estrous cycle (days 1 to 4), synchronization was impaired by the presence of newly emerging follicles incapable of ovulating in response to the first GnRH dose. These growing follicles had an increased chance of reaching atresia before $PGF_{2\alpha}$ was given. Later in the estrous cycle (day 10 and beyond), the presence of a functional DF at the time of first GnRH dose varied depending on follicular wave pattern of the cow and on time of follicular emergence. Moreover, it was possible to encounter spontaneous luteolysis triggered by endometrial $PGF_{2\alpha}$ during this period. Characterization of the ovarian events that take place when Ovsynch is started at different stages of the estrous cycle indicates that the main causes of synchronization failure were (1) lack of ovulatory response to the first GnRH, (2) atresia of the DF before $PGF_{2\alpha}$ administration, and (3) spontaneous luteolysis between administration of the first dose of GnRH and of $PGF_{2\alpha}$.[18] As previously stated, early atresia of the DF before $PGF_{2\alpha}$ administration was primarily a function of lack of ovulatory response to the first GnRH dose. For example, a 3-day-old follicle at the time of first GnRH dose (which probably would be the oldest not capable of ovulating[19]) would be 10 days old at the time of $PGF_{2\alpha}$ administration and already undergoing atresia. Therefore, at the time of the second GnRH dose, another follicular wave could be emerging, and the follicle destined to be dominant would be too young to respond to the final GnRH dose with ovulation. Cows in the latter situation probably would be in standing estrus 3 to 5 days after the second GnRH dose. In addition, cows with spontaneous luteolysis during the time from the first GnRH dose to $PGF_{2\alpha}$ administration probably would be in late stages of the estrous cycle and undergoing natural luteolysis before $PGF_{2\alpha}$ was given. Such cows probably would be in estrus from 1 day before to 1 day after $PGF_{2\alpha}$ administration. In either case, cows would not have a synchronized ovulation in response to the second GnRH injection and probably would not conceive with the timed AI.

Because synchronization to Ovsynch depends primarily on the ovulatory response to the first GnRH, followed by emergence of a new follicular wave,[18] several studies tried to develop presynchronization strategies to ensure presence of a functional mature DF at the time of the first GnRH of Ovsynch.[20–24] The most popular strategies were based on the use of $PGF_{2\alpha}$ at specific times before Ovsynch was started. The absence of consistent synchronization results probably was due to the fact that $PGF_{2\alpha}$ controls only the lifespan of the CL, which has little effect on follicular development. Thus, stage of follicular development at the beginning of Ovsynch probably would have too much variation if it was presynchronized only with $PGF_{2\alpha}$-based programs.

For instance, Momont and Seguin demonstrated dramatic differences in time, and variation in time, to estrus with $PGF_{2\alpha}$ treatment of cows at day 7 versus day 10 or 11 of the estrous cycle. Treatment at day 7 of the estrous cycle resulted in fewer days to estrus and less variation in time to estrus compared with treatment at days 10 to 11. This is explained by the difference in stage of follicular development between the two groups—a greater percentage of cows have mature DFs at day 7 than at days 10 to 11. Thus, treating cows with $PGF_{2\alpha}$ at random stages of the estrous cycle will not ensure synchrony of follicular development. Moreover, none of the presynchronization studies provided data on follicular events throughout presynchronization and Ovsynch to assess synchronization of follicular development.

Heifers are poorly synchronized when treated with Ovsynch (50%–60% SR).[2] This is the key reason why heifers treated with Ovsynch have significantly lower CR compared with heifers bred to detected estrus (35% versus 74%, respectively; $P < 0.01$).[2] The physiologic events underlying such poor synchronization can be interpreted

as an exacerbation of what happens in some of the cows that do not synchronize. Therefore, the synchronization situation in heifers may be used as a model to illustrate the underlying ovarian physiology of some nonsynchronizing cows.

Newly emerging follicles grow more rapidly[15] and for a shorter period[10,11] in heifers than they do in lactating cows. Thus, a DF in heifers can be expected to reach atresia sooner. Ovsynch-treated heifers that respond to the first GnRH dose may undergo follicular turnover of the new ovulatory DF before $PGF_{2\alpha}$ administration. If a DF from a new wave becomes atretic before the $PGF_{2\alpha}$ is given, an emerging follicular wave, rather than a mature DF, probably would be present at the time of the final GnRH injection. Consequently, synchronized ovulation to the final GnRH dose of Ovsynch is unlikely—and so is conception.

PROTOCOL COMPONENTS AND STRATEGIES

Interval between First GnRH Dose and $PGF_{2\alpha}$ Administration

Effects of Increasing Time between First GnRH Dose and $PGF_{2\alpha}$ Administration

Two potential scenarios emerge when the time from first GnRH dose to $PGF_{2\alpha}$ administration is increased to greater than 7 days: The first is that extending this period may allow a greater number of DF to reach atresia. As previously explained, this may result in a new follicular wave and the absence of a DF at the final GnRH dose. Thus, no ovulation would occur. Cows would then have continued follicular growth and ultimately estrus and ovulation 2 to 4 days after the final GnRH dose. As a second possibility, extending the time may increase the age of the DF at the time of $PGF_{2\alpha}$ administration and final GnRH injection. This could result in a persistent-type DF.

Treatments based on maintenance of subluteal levels of progesterone (P_4) (1–2 ng/ml) for an extended period are known to cause prolonged growth and dominance of the DF. This follicle is described as a persistent DF.[25–29] When the persistent DF is allowed to ovulate, fertility is decreased when compared with that of younger ovulatory follicles.[27,28,30–32] It was proposed that the negative effect of prolonged growth and dominance of the ovulatory follicle on fertility is associated with the hormonal environment in which the follicle is induced to persist. Subluteal levels of P_4 during a prolonged period cause increased frequency of LH pulsatility[33,34] and prevents a preovulatory LH surge from occurring.[28,32,35,36] As a result, the oocyte resumes meiosis while still contained in the follicle and starts undergoing premature nuclear maturation dissociated from follicular maturation and ovulation. Histologic characteristics of these persistent DFs indicate that the oocyte undergoes early germinal vesicle breakdown and continues a progression through the cell cycle toward metaphase I or II.[31,37] By the time of ovulation of the persistent follicle, the oocyte has already matured and aged, resulting in lower fertility, which may be explained by either low fertilization rates[38,39] or high early embryonic mortality,[40] or both. Also, persistent follicles are known to maintain a high and sustained production of estradiol, which may alter intrafollicular, oviductal, and uterine environments, thereby compromising sperm or oocyte transport and embryonic development.[26–28,31,32,37]

Follicular wave pattern and fertility. It is widely accepted that ovarian follicles develop primarily in two- or three-wave patterns throughout the estrous cycle of cattle[4,42,43] (Fig. 38-2). Lactating dairy cows generally have two waves of follicular growth per cycle.[10,11,44–46] In either wave pattern, the DF of the final wave is the one intended to ovulate. The mean interovulatory interval is shorter for two-wave versus three-wave cows (approximately 20 days versus 22–23 days, respectively), and the emergence of the ovulatory wave occurs earlier for two-wave than for three-wave cows (approximately day 11 versus day 16 of the cycle), leading to different lifespans of the ovulatory follicles for each wave pattern. At the time of ovulation, therefore, a difference in age can be observed between the preovulatory follicle coming from a two-wave (9 to 10 days) versus a three-wave (6 to 7 days) pattern of follicle development.[41,43] Thus, the two-wave pattern that pre-

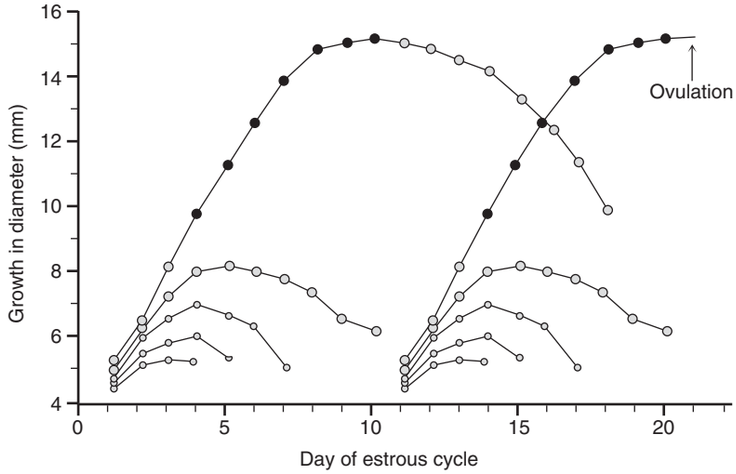

Fig. 38-2 Diagram of the growth in diameter (mm) of follicles during a 21-day bovine estrous cycle. The cow had two follicular waves during this estrous cycle. The black points indicate when a dominant follicle (DF) probably is responsive to GnRH.

dominates in lactating dairy cows generally is characterized by a larger and older DF reaching ovulation compared with that in three-wave patterns.[1,44] Moreover, second-wave preovulatory follicles were found to be approximately 3 days older than those of a third wave when the interval from emergence to estrus was measured.[44] This difference in lifespan between second-wave and third-wave ovulatory follicles was explained by a longer period of follicular dominance, rather than by the interval of follicle growth from emergence to dominance, which remained the same in both cases.[44]

As previously stated, the ovulatory follicle of a two-wave cycle is older than that of a three-wave cycle. By analogy with the effect of prolonged dominance of a persistent follicle, the older age of a second-wave ovulatory follicle could be expected to have a negative impact on fertility. If most dairy cows have two waves, then a high proportion of the population could be expected to be ovulating an older DF, thus providing a possible explanation of the low CR problem in lactating dairy cows. In applying this concept to Ovsynch, the established 7-day interval may be limiting the number of cows that ovulate an aged follicle. This may be the reason why fertility of cows that synchronized exceeds fertility of cows inseminated after a detected estrus. Decreasing the age of the follicle at the time of the final GnRH so that it is more like a three-wave follicle may offer greater improvements. Only a few studies compared fertility between three- and two-wave cows.[44,46,47] There may be significant trends toward greater CR in three- than in two-wave cows. Unfortunately, sufficient numbers of three-wave cows probably were difficult to procure for these studies, because most lactating dairy cows have two follicular waves during a normal estrous cycle.[48]

Effect of Duration of Dominance of Ovulatory Follicle on Fertility

An even stronger association is likely between fertility and the length of ovulatory follicle lifespan or duration of physiologic dominance, than between fertility and the number of follicular waves. In one study, the interval from emergence of the ovulatory follicle to estrus was approximately 1 day shorter in cows diagnosed as pregnant compared with cows diagnosed as not pregnant (7.8 ± 0.2 days versus 8.6 ± 0.2 days, respectively; $P < 0.01$), regardless of whether they had two or three follicular waves.[44] A key result from the same study was a significant inverse relationship between duration of dominance and CR. The longer the ovulatory follicle remained in the ovary waiting for the ovulation trigger, the lower the probability of establishing a pregnancy.

Furthermore, in a recent study that compared follicle development between lactating cows and nulliparous heifers,[11] heifers had a 1.2-day shorter duration of dominance of the ovulatory follicle when compared with cows. The relevance of this issue becomes clear when the CR difference between these two groups is considered. It may be possible to reduce the number of cows that would ovulate an old follicle. The only possible way to accomplish this, however, is to reduce the time from the first GnRH dose to $PGF_{2\alpha}$ administration.

Effects of Reducing the Time between First GnRH Dose and $PGF_{2\alpha}$ Administration

Altering the time from first GnRH dose to $PGF_{2\alpha}$ administration probably could affect the maturity level of the ovulatory follicle at the time of the final GnRH dose. Follicles that are induced to ovulate before reaching physiologic maturity may be less fertile. Several studies demonstrated the relationship between smaller follicular size at the time of induced ovulation with GnRH and lower CR.[9,49,50] In a recent study from our laboratory, cows treated with Ovsynch that ovulated to a GnRH-induced LH surge with ovulatory follicles less than 12 mm in diameter had lower CR compared with ovulatory follicles 12 mm or larger (27% versus 36%; $P < 0.01$) and comprised nearly 34% of the cows in the study ($N = 1424$).[9] It is likely that these follicles were not allowed to reach peak physiologic maturity.

Compromised oocyte quality and subsequent inadequate luteal function are possible reasons why reduced age, size, or physiologic status of follicles reduces CR. Lower circulating P_4 concentrations in the subsequent cycle[51] can impair embryo recognition, development, and implantation. Another possibility could be related to a nonsynchronized, and hence incorrect, timing of endocrine signals controlling final follicle and oocyte maturation, leading to ovulation of an immature oocyte.[52] Reduced estradiol concentrations due to a younger DF also can alter uterine and oviductal environment, affecting either gamete or embryo survivability.[16,29,43,52,53]

Physiology and Regression of an Early Corpus Luteum

The main requirement for an Ovsynch-type program that intends to increase SR and decrease lifespan and duration of dominance of its ovulatory follicle is regression of an early CL (5 days old or less; day 0 = estrus). To our knowledge, reducing the interval between the first GnRH dose and $PGF_{2\alpha}$ administration to less than 7 days has not been reported in lactating dairy cows. CL regression, however, was consistently attained after a 6-day interval between first GnRH and $PGF_{2\alpha}$ administration in dairy and beef heifers and beef cows.[55,56] Moreover, consistent regression of a 4- or 5-day-old CL is possible with repeated injections of $PGF_{2\alpha}$.[57–59]

Experiments carried out in the 1970s and 1980s provided evidence that a CL was refractory to exogenously induced luteolysis during the first few days after being formed. A single treatment with $PGF_{2\alpha}$ was unable to initiate CL regression within the first 4 days of the cycle,[57,60–62] and even though some sensitivity to a luteolytic dose of $PGF_{2\alpha}$ can be observed in 5-day-old CLs, this response was not consistent until the CL reached an age of approximately 7 days.[62–64] It is clear that this lack of early CL response cannot be attributed to lack of $PGF_{2\alpha}$ receptors, because a CL as young as 2 days already expresses $PGF_{2\alpha}$ receptors.[65–67] Despite several mediators proposed to explain this event,[68–73] the mechanisms underlying early luteolytic resistance of a CL are still not clear.

Nevertheless, some evidence supports a cumulative effect of frequently repeated injections of $PGF_{2\alpha}$ on early CL sensitivity to luteolysis. First, after twice-daily (12-hour interval) injections of $PGF_{2\alpha}$ on days 3 and 4 of the cycle (day 0 = estrus), the newly formed CL regressed, leading to shortening of the estrous cycle and manifestation of precocious estrus in Holstein heifers.[57] Second, in dairy heifers receiving a daily injection of $PGF_{2\alpha}$ from days 3 to 7 of the cycle, plasma P_4 levels remained low, and manifestations of estrus appeared.[59] Third, even less intensive treatments, consisting of a daily dose of $PGF_{2\alpha}$ on two consecutive days within days 0 to 5 of the estrous cycle, produced early luteal regression in a proportion of heifers.[58] Even in the heifers that did not show a complete luteolytic response, either a single injection of $PGF_{2\alpha}$ on day 4 or twice-daily injections on days 2 and 3 of cycle were enough to depress peripheral P_4 concentrations throughout the cycle.[57] Moreover, at the molecular level, repeatedly treated day 4 CLs showed up-regulation of $PGF_{2\alpha}$ synthase in luteal cells.[74] Therefore, it appears that a cumulative $PGF_{2\alpha}$ stimulus, together with a certain maturation level of the CL, may be associated with increased early CL sensitivity to an intensive luteolytic treatment in heifers. These results led us to postulate that early CL responsiveness to $PGF_{2\alpha}$ may be dependent on the magnitude or duration (or both) of the stimuli to which it is exposed, which can be tested through a dose-response study.

In this context, it is suitable to consider the type of luteolytic agent used to induce the demise of a CL at an earlier stage of the cycle (day 5 or 6). Two $PGF_{2\alpha}$ products currently are available for use in cattle: (1) dinoprost, a natural tromethamine salt of $PGF_{2\alpha}$, which is available as either Prostamate or Lutalyse; and (2) cloprostenol, a synthetic prostaglandin analogue, commercially available as Estrumate. These two products differ in their pharmacokinetic properties when administered to cows: Dinoprost has a short half-life ($t_{1/2}$) of 7 to 18 minutes,[75–77] whereas cloprostenol seems to be more resistant to endogenous metabolism, maintaining higher circulating concentrations for a longer period ($t_{1/2}$ = 3 hours).[57] Nevertheless, on the basis of findings in several trials performed to compare luteolytic efficacy of one dose of dinoprost or cloprostenol in cattle,[77,78,80–82] there seems to be no difference between them. All of these experiments, however, tested for differential luteolytic efficacy either at random stages of the estrous cycle or when a mature $PGF_{2\alpha}$-sensitive CL (8 days old or less) was present. None of these experiments tested the question of differential luteal regression using dinoprost versus cloprostenol at the critical period of early CL refractoriness. In considering the effect on early CL regression of both repeated $PGF_{2\alpha}$ treatments in a short time and a longer $t_{1/2}$ of cloprostenol in circulation, it seems reasonable to expect differential luteolytic efficacy of dinoprost and cloprostenol in this critical early period of the cycle.

Further physiologic reasons to support the relevance of a cumulative $PGF_{2\alpha}$ effect during luteolysis arise from a potential local autoamplification luteolytic pathway within the CL. There is evidence for hormonal interactions leading to luteal regression not only through an endocrine pathway but also by means of paracrine/autocrine mechanisms. At luteolysis, endometrial $PGF_{2\alpha}$ reaches the ovaries through a countercurrent mechanism and induces demise of the CL, both functionally, by cessation of P_4 production, and structurally, by involution of luteal tissue.[83–86] Additional evidence indicates that luteal secretion of $PGF_{2\alpha}$[74,86–88] may be a potential local amplification signal that would augment the luteolytic effect of uterine $PGF_{2\alpha}$. Although the physiologic significance of this local pathway is not clear, an intraluteal autoamplification pathway triggered by small amounts of uterine $PGF_{2\alpha}$ may be important to complete luteolysis.[89] The relevance of a local mechanism becomes apparent when one considers that more than 99% of uterine $PGF_{2\alpha}$ is metabolized and removed from circulation after a single passage through the liver and the pulmonary bed.[90] Thus, in the few seconds that it takes for portal blood to go through the lungs and be redistributed again in the systemic circulation, $PGF_{2\alpha}$ concentrations are reduced to less than 1%. The relevance of a potential local autoamplification pathway to our approach is related to the importance of a cumulative effect of the stimuli during luteolysis.

When developing Ovsynch, Pursley and collaborators[15] decided to establish a 7-day interval between the first GnRH of the program and $PGF_{2\alpha}$. A 7-day period was assumed to be an appropriate interval to allow for follicular development and CL maturity at the time of induction of luteolysis. It was never formally tested, however, whether the 7-day interval assumption was actually optimal for SR in Ovsynch-treated cows. Therefore, the question of whether SR can be improved by reducing the interval from first GnRH to $PGF_{2\alpha}$ remains unanswered. This reduced interval may allow greater control of the maturity level of the DF at the time of ovulation. We believe that an ovulatory follicle of reduced lifespan and duration of physiologic dominance may have a positive impact on fertility in dairy cows if this follicle is allowed to reach maturity. A synchronization program capable of consistently and accurately generating an ovulatory follicle of such characteristics will provide the opportunity to test this novel question. A significant by-product of the development of such a program could be a substantial improvement in SR, with indirectly improvements in CRs with Ovsynch.

Interval from $PGF_{2\alpha}$ Administration to Final GNRH Dose

A recent study from our laboratory suggests there is very little flexibility in time from administration of $PGF_{2\alpha}$ to the final treatment with GnRH in Ovsynch.[24] In order to control time of the LH surge so that time of ovulation can be controlled, the final GnRH administration of Ovsynch must be given before a spontaneous LH surge to control time of AI. Studies suggest that a spontaneous LH surge can occur as early as 36 hours after a $PGF_{2\alpha}$-induced CL regression, with the greatest percentage occurring after 48 hours. We tested the effect of inducing the LH surge with GnRH at 0, 12, 24, 36, and 48 hours after $PGF_{2\alpha}$ administration. Increasing the time between the decline of P_4 until the LH surge increased CR. This study yielded direct evidence of a positive association with length of time

from $PGF_{2\alpha}$ to GnRH and reduced short luteal phases in the subsequent estrous cycle. Other possible explanations for an increase in CR as time increased from luteal regression to the LH surge include improved oviduct contractility and oocyte transport and greater sperm survival and transport.

Lengthening the time from $PGF_{2\alpha}$ to the final GnRH is gaining attention in many herds, but no published data on this strategy are yet available. When GnRH is administered more than 48 hours after $PGF_{2\alpha}$, the chance of inducing an LH surge with the final GnRH diminishes with time, and so does the control over the time of ovulation. The reason for this is because cows begin having spontaneous LH surges at approximately 40 hours after $PGF_{2\alpha}$ administration. If all cows that had spontaneous LH surges were detected in estrus before the final GnRH, then timing of AI could be suited to the time of the onset of estrus. Thus, with use of this lengthened interval, it is critical to utilize and enhance estrus detection between $PGF_{2\alpha}$ and the final GnRH dose.

Timing of Artificial Insemination Relative to Final GnRH Dose in Lactating Holstein Cows

Data from Pursley and colleagues[91] suggest that AI at 16 hours after the final GnRH injection, when administered 48 hours after $PGF_{2\alpha}$ administration, results in the highest CR, compared with AI at 0, 8, 24, and 32 hours after the final GnRH dose. This study clearly indicated that AI after ovulation severely depressed CR and increased pregnancy losses between 28 and 56 days after AI. A study just completed in our laboratory indicates that AI at 8 hours before the final GnRH (equivalent to approximately 36 hours before ovulation) reduced CR nearly 30%, compared with AI at 16 hours after the final GnRH (32% versus 23%).[9] Of interest, both studies demonstrated a significant increase in female calves when AI was done at least 28 hours before ovulation.

FUTURE DIRECTIONS WITH OVSYNCH

Clearly, some aspects of the initial development of Ovsynch were novel, including the use of GnRH to initiate a new follicular wave and a second dose of GnRH to induce ovulation of the DF from that new wave. The development of Ovsynch, however, was not focused on improving the competency of the ovulatory follicle. Studies are needed to optimize the timing of all injections to ensure maximal development of a DF of reduced lifespan and duration of dominance. An increase in CR from improving SR alone could amount to 6% more pregnant cows every week on dairy farms. If it is possible to increase the fertility of the ovulatory follicle, then much greater increases in CR could be achieved. Because Ovsynch currently is in place in many dairy management systems across the United States and the world, key improvements could be implemented in a straightforward and trouble-free fashion. Increases in pregnancy rate due to improvements in each aspect of Ovsynch could be expected to have several benefits: (1) increased milk production per cow due to maintenance of a greater proportion of cows in early lactation, (2) decrease in involuntary culling due to either a lack of pregnancy or pregnancy too late in lactation, and (3) increase in the number of replacements born each year. These positive changes in herd management could increase dairy farm profit substantially.

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CHAPTER 39
Pregnancy Diagnosis

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Since the beginning of civilization, animal owners have been interested in determining whether or not conception has taken place, and various clinical signs and superstitions have been used for millennia to diagnose pregnancy.[1] Over time, a number of more accurate methods for determination of pregnancy in cows and other female domestic animals have been developed, including observation, physical examination, chemical tests, and use of electronic instruments.

INDICATIONS

The purpose of examining cows for pregnancy is not to detect those that are pregnant, but to detect those that are not pregnant so that they can be inseminated again or culled from the herd. For profitable production, dairy cows should calve for the first time at approximately 24 months of age and should deliver subsequent calves at intervals of approximately 13 to 13.5 months. Thus, dairy cows should conceive within approximately 4 months or less of calving. Cows that are found to be not pregnant can be treated for any abnormalities of the reproductive tract discovered during the examination, observed closely for signs of spontaneous estrus, or treated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to predictably shorten the time until return to estrus.

An ideal test would accurately detect pregnancy before the first expected estrus after insemination (about 21 days) so that the cow could be reinseminated without further loss of time. Unfortunately, no tests are currently available that are practical to use and allow detection of pregnancy in cows before the first expected estrus. Most methods for pregnancy diagnosis are capable of detecting pregnancy between 25 and 40 days after conception, and most authors have traditionally recommended that nonpregnant cows be identified before the second expected estrus after insemination.[2] In a recent prospective study, however, cows that were diagnosed as pregnant by palpation between 30 and 36 days after breeding were found to have a 2-week longer calving interval than that observed in cows examined for pregnancy later.[3]

The frequency of embryonic death in cattle is high during the first months of pregnancy, perhaps because of loss of abnormal embryos or failure of maternal recognition of pregnancy.[4] Thus, cows that are diagnosed as pregnant soon after insemination are more likely to suffer embryonic death and return to estrus, to be found not pregnant at a subsequent examination, or to fail to deliver a calf at the expected time than are cows in which pregnancy is diagnosed later. Owners frequently misinterpret this course of events and conclude that the test for pregnancy was inaccurate (i.e., the cow was not pregnant at the time of examination) or that the test affected the embryo and directly or indirectly resulted in termination of pregnancy. After approximately 60 days, fetal death rates are low, and in most, but not all, cows that are found to be pregnant after this time, the pregnancy proceeds to term with birth of a calf.

Beef cows usually are examined for pregnancy when their calves are weaned at 6 to 7 months of age, although in intensively managed herds, individual cows can be examined earlier. In most management systems, nonpregnant cows are culled from the herd to save the cost of maintaining nonproductive animals, but in herds that have spring and autumn calving seasons, cows sometimes are moved to the other group and given a second opportunity, although this decision may be difficult to justify economically. Although the cost of maintaining a nonproductive beef cow is variously estimated to be between $250 and $400 per year, a survey of herd owners in 18 states revealed that only 17.7% examine cows and that 15.9% examine heifers for pregnancy.[5]

MANAGEMENT METHODS FOR PREGNANCY DIAGNOSIS

As described later on, palpation of reproductive structures per rectum (probably to be replaced by ultrasound examination in the foreseeable future) has been the customary method of pregnancy diagnosis in cattle. Some cattle owners, however, may rely on the history, or on clinical signs that can be observed, for a presumptive diagnosis of pregnancy.

Exposure to a Bull or Artificial Insemination

A history of cohabitation with a bull, the observation of mating, or artificial insemination may be considered by some owners to be sufficient evidence that a cow has become pregnant. Although fertilization rates in cows typically are high, however, only approximately 50% of inseminations result in a detectable pregnancy. Conversely, unobserved, unplanned, or unrecorded matings are not uncommon, and cows frequently are presented with a complaint of infertility, only to be found pregnant when examined. Thus, history is not a reliable indicator of pregnancy status and sometimes may be deceptive.

Cessation of the Estrous Cycle

Bovine embryos signal their presence around 15 to 17 days after ovulation: The corpus luteum is maintained,

and the maternal estrous cycle is suspended. Thus, failure of a cow to return to estrus at approximately 18 to 24 days after mating suggests that conception has occurred. In fact, the most common cause of failure of cows to have normal estrous cycles is pregnancy.[6]

In beef herds in which natural service is used, perceptive managers may observe that a greater-than-expected number of cows return to estrus after mating. This situation suggests an infertile bull, the presence of a venereal infection, or some other cause of infertility, and an opportunity exists to take corrective action before the breeding season ends. Conversely, undernutrition is a common cause of anestrus in lactating beef cows, and the observation that few cows return to estrus after the first few weeks of the breeding season may mislead less astute managers to believe that cows have become pregnant when they are in fact not cycling.

Failure of a dairy cow to be detected in estrus at approximately 3 weeks after insemination is viewed as a favorable event by managers, and anestrous cows are assumed to be pregnant. Unfortunately, although the efficiency of estrus detection varies among observers and among dairy farms, it generally is lower than desirable, so this approach is not sufficiently accurate to be of use in diagnosis of pregnancy. Most dairy reproductive herd health programs are based on the practice of examining cows that have not been observed in estrus after insemination as indicated by some other method (most commonly rectal palpation) that more accurately differentiates between pregnant and nonpregnant cows.

A few pregnant cows show mild to conspicuous signs of estrus and may be mistakenly thought to be nonpregnant. Artificial insemination of pregnant cows may result in abortion if the insemination instrument is passed completely through the cervical canal and the fetal membranes are disrupted.[7]

Metestrus Bleeding

A sanguineous vaginal discharge is common in cows 24 to 48 hours after estrus and is the result of hemorrhage from the caruncular capillaries due to the rapid decline in estrogen concentration that follows ovulation. Some owners subscribe to the notion that metestrus bleeding is a sign of conception failure; however, metestrus bleeding is observed in nearly all cows and heifers and is not an indication of whether conception occurred. If metestrus bleeding is observed in a cow that was not seen in estrus a few days previously, it is implied that estrus was unobserved and that the animal is not pregnant.

PALPATION PER RECTUM

Palpation of the reproductive tract through the rectal wall (rectal palpation) has been used for diagnosis of pregnancy since the early 1900s and has been the customary method used in cattle for more than 50 years.[8–11] Depending on the skill of the examiner and the age and size of the dam, rectal palpation is useful to diagnose pregnancy as early as day 30 and thereafter until term. Although a number of changes occur in the size, texture, location, and content of the uterus during pregnancy, four *positive* signs of pregnancy that are detectable by rectal palpation are recognized:

- Palpation of the fetal membrane slip
- Palpation of the amniotic vesicle
- Palpation of placentomes
- Palpation of the fetus

The examiner must detect at least one of these four signs before declaring the cow pregnant.

Fetal Membrane Slip

The examiner can detect the chorioallantois within the lumen of the pregnant uterus by compressing the uterine horn between the thumb and forefingers, lifting the uterus, and then allowing the horn to slowly "slip" from the grasp. If the cow is pregnant, the chorioallantois can be felt to slip through the fingers just in front of the uterine wall. This membrane slip can be felt in the pregnant uterine horn as early as 30 days of pregnancy and can be reliably detected by day 35. The fetal membrane slip can be detected in the nongravid horn by approximately day 70 of pregnancy. During early pregnancy, the fetal membranes are thin, and a delicate touch and some experience are required to recognize this sign of pregnancy.

Amniotic Vesicle

The amnion contains the developing conceptus and the amniotic fluid and is palpable as early as 28 days after conception in heifers and by 32 to 35 days in pluriparous cows. The vesicle is recognized as a nearly spherical, turgid, fluid-filled structure that is approximately 1 cm in diameter at 28 days and increases in size as pregnancy advances. The amniotic vesicle is detected by encircling the uterine horn with the examiner's thumb on one side and the fingers on the other. The vesicle is free-floating within the uterus but most commonly is found at the cranial edge of the intercornual ligament. The amniotic vesicle becomes progressively less turgid and is difficult to recognize by about day 65 of gestation. At that time, the vesicle softens and the fetus becomes palpable. In a bovine conceptus, the heart is external until approximately day 42; therefore, caution must be exercised in attempting to detect early pregnancies, and undue pressure must not be applied to the amniotic vesicle because rupture of the embryonic heart or other fragile organs may result. Intentional rupture of the amniotic vesicle has been used as a method to provoke abortion in cattle.[12]

Placentomes

In ruminants, cotyledons of the fetal placenta produce villi that project into crypts of maternal caruncles to form placentomes. Seventy-five to 120 maternal caruncles arranged in two dorsal and two ventral rows are present in the uterus of cows. Placentomes begin to form early in gestation and are of sufficient size to be palpable by 75 to 80 days. The size of placentomes varies with the stage of gestation and their location in the uterus. Placentomes

are progressively larger near the middle of the gravid horn and are smaller at the cervical and ovarian poles. They are most consistent in size immediately cranial to the cervix and are palpated at that location to estimate the stage of pregnancy. Placentomes are identified by grasping a longitudinal fold of the uterine wall and rolling it between the thumb and fingers. In more advanced pregnancies, the examiner can palpate placentomes by passing a flattened hand over the uterine wall.

Placentomes remain palpable for a variable time after death of the conceptus, and detection of their presence may, in a few instances, result in a false positive diagnosis of pregnancy. A false positive diagnosis of pregnancy also can occur when an ovary is mistaken for a placentome consistent in size with a 120- to 180-day pregnancy. The examiner can easily avoid this mistake by identifying at least three placentomes before declaring the animal pregnant (there are only two ovaries).

A false negative diagnosis is possible during the fifth to eighth month of pregnancy, when the uterus is completely descended and may be out of reach, especially in large, deep-bodied cows, or to examiners with short arms. This mistake can be avoided if the examiner searches for other signs of pregnancy before declaring the cow pregnant, or positively identifies a normal, nongravid uterus before declaring the animal nonpregnant.

Fetus

The fetus becomes palpable at approximately 65 days, when the amniotic membrane loses its turgidity, and remains theoretically palpable for the balance of gestation. In the early stages of gestation, the fetus can be grasped directly. Later, the fetus is detected by ballottement: The examiner sets the fetal fluids in motion by rocking the hand against the uterine wall and recognizes the fetus as it rebounds against the hand. The fetus is easily palpable as a free-floating, firm object within the fluid-filled uterus during the first 4 months of gestation. As pregnancy advances, increased weight of the fetus and fluid pulls the uterus ventrally and cranially until the fetus comes to rest on the abdominal floor during the fifth and sixth months. Continued growth of the fetus positions it closer to the maternal pelvis during the last trimester (period of ascent), so that palpation of the fetus is facilitated. Roberts[11] has estimated that it is possible to palpate the fetus in greater than 95% of cows during the third and fourth months of gestation, in 40% to 70% of cows during the fifth and sixth months, in 80% at 7 months, and in greater than 95% during the eighth and ninth months.

False negative diagnoses are most likely if palpation of the fetus is relied on for diagnosis of pregnancy between the fifth and seventh months. Mistakes can be avoided if the cow is examined for other signs of pregnancy or if a nongravid uterus is identified before the animal is declared not pregnant.

False positive diagnosis is possible if another structure is mistaken for a fetus. Some of the normal maternal structures that may be incorrectly perceived as a fetus are the dorsal sac of the rumen and the left kidney. Among the abnormal structures that have been mistaken for a portion of the fetus are ovarian and uterine tumors, enlarged lymph nodes, adhesions and connective tissue secondary to previous surgical or obstetric trauma, and necrosis of abdominal fat. Mummified fetuses can be mistaken for a normal pregnancy if an examination is perfunctory. Although a mummified fetus can approximate the size of a viable fetus, mummies are recognized by a lack of uterine fluid and absence of fetal membranes and placentomes.

Supporting Signs of Pregnancy

Several palpable changes in the reproductive tract suggest pregnancy but can have other causes. Thus, these changes constitute supporting but not specific signs of pregnancy.

Asymmetry of the uterine horns. Examinations for pregnancy should always begin with palpation of both uterine horns to detect differences in their size. In very early pregnancies, the horns may be nearly identical in size, but as gestation advances, the pregnant horn enlarges. Asymmetry often is first detectable in the cranial portion of the gravid horn. If a difference in the diameter of the uterine horns is detected, the larger horn can be examined for presence of an amniotic vesicle or fetal membrane slip. Conditions other than pregnancy that potentially may cause enlargement of one uterine horn are pyometra, mucometra, and delayed postpartum uterine involution. In none of these conditions, however, is an amniotic vesicle or fetal membrane slip detectable.

Resilience and fluctuance of the uterine wall. As the gravid uterine horn fills with fluid, the wall thins and assumes a characteristic texture of resilience and fluctuance. With some experience, the texture of the uterine contents and the uterine wall typically associated with pregnancy will give the examiner an early indication that the cow is pregnant. This impression is confirmed by detection of at least one of the four positive signs of pregnancy.

Fixation of the cervix. The cervix can be lifted and the uterus retracted until approximately day 65 to 70. By about day 90, the weight of fluid within the uterus becomes sufficient to prevent the examiner from easily lifting the cervix, and the organ is said to be "fixed." Although fixation of the cervix commonly is associated with pregnancy, abnormal accumulations of fluid within the uterus such as those associated with pyometra and mucometra also can cause cervical fixation. Adhesions between the reproductive tract and other pelvic or abdominal organs such as those that follow cesarean section or obstetric trauma also can prevent retraction of the cervix. Uterine or ovarian tumors may be large enough to pull the reproductive tract into the abdominal cavity and also prevent retraction of the cervix.

Hypertrophy of the middle uterine artery. As gestation advances, blood supply to the uterus is increased to meet demands of the developing fetus. The middle uterine artery lies in the broad ligament, which allows it to be freely movable for a distance of 10 to 15 cm. An increase in size of the uterine artery supplying the gravid horn is detectable in heifers as early as day 60 to 75 and

in cows by day 90. Increased size and thinning of the wall of the artery lead to the unique palpable characteristic of fremitus. Fremitus is first palpable in the artery supplying the gravid horn and later the nongravid horn. The perception of fremitus may be enhanced by applying slight pressure to the artery, but it disappears if the artery is occluded. Although fremitus is a sign often associated with pregnancy, it persists for several days after abortion or parturition and also accompanies conditions other than pregnancy that increase blood flow to the uterus.

Ovarian changes. Every examination of the reproductive tract begins with a thorough examination of both uterine horns, and the ovaries usually are not examined until after the operator has determined that the cow is not pregnant; however, in some cases it may be useful to examine the ovaries if the operator is uncertain after palpating the uterus. Cows depend on progesterone secreted by the corpus luteum to maintain pregnancy throughout gestation. Transuterine migration of embryos is uncommon in cows; thus, a corpus luteum is almost always present in the ovary ipsilateral to the pregnant uterine horn. Consequently, the presence of a corpus luteum ipsilateral to an enlarged, fluid-filled uterine horn suggests pregnancy. Conditions such as pyometra and other abnormalities of the uterus, however, also cause persistence of corpora lutea and accumulation of fluid within the uterine horns. Furthermore, the ability of most clinicians to detect a functional corpus luteum by rectal palpation is inadequate; thus, failure to perceive a corpus luteum should not be the sole basis for declaring a cow not pregnant.[13] One of the positive signs of pregnancy must be identified before the cow is declared pregnant or their absence determined with certainty before the cow is declared not pregnant.

In some instances, it may be desirable to examine cows around the time of the first expected estrus (about 21 days) after insemination. If the cow is not pregnant, uterine and ovarian changes typical of approaching estrus such as increased uterine tone, a regressed corpus luteum and developing follicle or recent ovulation, and a clear mucous vulvar discharge are to be expected. If the cow is pregnant, no positive signs of pregnancy will be detectable, but a mature corpus luteum will be palpable in one of the ovaries.

Estimation of the Stage of Gestation

The stage of gestation can be estimated on the basis of palpable characteristics of the uterus and fetus. Estimation of the stage of gestation is most accurate during the first half of pregnancy. In early pregnancies, stage of gestation can be estimated on the basis of the size of the gravid horn and the diameter of the amniotic vesicle. In more advanced pregnancies, age of the fetus is estimated on the basis of determination of the size of placentomes at the base of the pregnant uterine horn, size of the fetus, fetal crown-to-nose length,[14] position of the uterus, and size of the middle uterine artery.[10,11] Characteristic sizes at different stages of gestation are summarized in Table 39-1.

Accuracy of Rectal Palpation

Traditionally, most theriogenologists have regarded rectal palpation to be the definitive method for diagnosis of pregnancy and have assumed that its accuracy approaches 100%. Until recently, few methods other than slaughter have been available to test the accuracy of rectal palpation. When performed diligently and thoroughly, rectal palpation probably is the most practical and accurate method of pregnancy diagnosis available for use in cows, but clinicians should not undertake examinations for pregnancy until animals have reached the stage of gestation when a diagnosis of pregnancy is possible. With a moderate amount of experience, a reliable diagnosis of pregnancy is possible after day 35 to 39. Clinicians with less experience may wish to delay examinations for pregnancy until 50 to 55 days after breeding, when positive signs of pregnancy are more easily detected.

Despite the confidence of practitioners, false positive and false negative diagnoses are possible. Veterinarians occasionally suffer the indignity of an owner's report that a cow declared not pregnant aborted after administration of prostaglandin $F_{2\alpha}$ to induce estrus or was found to be pregnant at slaughter. Conversely, some cows declared pregnant return to estrus or fail to calve. A proportion of such cases can be explained by diagnostic error, but a majority probably are due to embryonic or fetal death that occurs after examination. The incidence of pregnancy loss is high during early gestation, and owners should be informed of that possibility.[15] In a recent study it was found that of the cows diagnosed as pregnant by rectal palpation, 3.4% returned to estrus and subsequently were inseminated, and a further 1.5% were found not to be pregnant when examined at a later date. Approximately 5% of cows diagnosed as not pregnant calved at a time consistent with having been pregnant when the diagnosis was made.[3]

Prudent clinicians observe the rules for pregnancy examination set forth by Zemjanis[10]:

- Pregnancy examination should always represent the first step of genital examination.
- No animal should be treated unless the operator is positive that the animal is non-pregnant.
- No animal should be pronounced non-pregnant unless the uterus has been retracted and both horns of the uterus have been palpated carefully throughout their entire length.
- A diagnosis of pregnancy should never be made unless the positive signs of pregnancy have been detected and recognized beyond doubt.

Safety of Rectal Palpation

For decades, pregnancy diagnosis was assumed to be safe for the dam and the fetus. Several reports have appeared in the literature, however, that suggest that rectal palpation is not an innocuous procedure and carries with it inherent risks for both dam and fetus.

Fetal Death

The possibility that specific methods of pregnancy diagnosis by rectal palpation may contribute to embryonic

Table **39-1**

Characteristics of Pregnancy in Cows

Gestation Length, d	Amniotic Vesicle Size, cm	Pregnant Horn Diameter, cm	Placentome Diameter, cm	Middle Uterine Artery Size, cm	Crown-To-Nose Length, cm	Crown-Rump Length, cm	Uterine Position	Example of Fetal Size	Characteristics of Fetus	Positive Signs of Pregnancy
30	0.8–1 (size of a pea)	2–4	—	0.4–0.6	—	0.8–1	Pelvic	—	Head and limb buds; placenta is not attached	Amniotic vesicle
40	2–3 (size of a plum)	4–6	—	0.4–0.6	—	1.75–2.5	Pelvic	—	—	Amniotic vesicle; fetal membrane slip
50	3.5–5	5–7	—	0.4–0.6	—	3.5–5.5	Pelvic	—	—	Amniotic vesicle; fetal membrane slip
60	6–7.5	6–9	—	0.4–0.6	—	6–8	Pelvic	Mouse	Claw buds and scrotum visible; palate and sternum closed; placenta attached; small placentomes visible	Amniotic vesicle softens; fetus; fetal membrane slip
70	—	7–10	0.5–0.75	0.5–0.7	1.5 (one finger width)	7–10	Uterine descent begins	—	—	Fetus; fetal membrane slip; placentomes
80	—	9–12	0.5–1	0.5–0.7 (fremitus)	3.5 (two finger widths)	8–13	Uterus descending	—	—	Fetus; placentomes; fetal membrane slip
90	—	10–13	1–1.5 (size of a dime)	0.5–0.7 (fremitus)	5.5 (three finger widths)	13–17	Uterus descending	Rat	Hair on lips, chin and eyelids; scrotum present	Fetus; placentomes

120	—	12.5–18	1.5–2.5 (size of a quarter)	0.7–0.9 (fremitus)	10.5 (wide as hand and thumb)	22–32	Uterus descending	Small cat	Fine hair on eyebrows; claws developed; horn pits present; epithelial plaques on amnion	Fetus; placentomes
150	—	18–23	2.5–4 (size of a half dollar)	0.7–1.0 (fremitus)	—	30–45	Uterus on abdominal floor	Large cat	Hair on eyebrows and lips; testes in scrotum; teats developing	Fetus (palpation may be difficult); placentomes
180	—	—	4–5	0.9–1.25 (fremitus)	—	40–60	Uterus descended	Beagle dog	Hair on inside of ear and around the horn pits, tip of tail, and muzzle	Fetus (palpation may be difficult); placentomes
210	—	—	5–7.5	1.25–1.5 (fremitus)	—	55–75	Ascent of the uterus begins	—	Hair on metatarsal, metacarpal, and phalangeal region of extremities and beginning on the back, long hair on tip of tail	Fetus; placentomes
240	—	—	6–9	1.25–1.7 (fremitus)	—	60–85	Ascending	—	Fine short hair all over the body; incisor teeth are not erupted	Fetus; placentomes
270	—	—	8–12	1.5–1.9 (fremitus)	—	70–100	Ascended; fetus readily palpable	—	Hair coat complete and long; fetus large; incisor teeth erupted	Fetus; placentomes

Data from Benesch F, Wright JG: *Veterinary Obstetrics,* p 29. Baltimore: Williams & Wilkins, 1951; Zemjanis R: In *Diagnostic and Therapeutic Techniques in Animal Reproduction,* 2nd ed, p 29. Baltimore: Williams & Wilkins, 1970; Roberts SJ: In *Veterinary Obstetrics and Genital Diseases (Theriogenology),* 3rd ed, p 14. Woodstock, VT: published by the author, 1986; and Ball L: In Morrow DA (ed): *Current Therapy in Theriogenology,* p 229. Philadelphia: WB Saunders Co, 1980.

and fetal death was raised by the report of a prospective study in which cows were examined for pregnancy by palpation of uterine fluctuation, palpation of the amniotic vesicle, and palpation of the fetal membrane slip.[16] In that study, it was found that palpation for uterine fluctuation alone in cows pregnant from 35 to 70 days was an accurate and safe method to detect pregnancy, but that detection of pregnancy by palpation of the fetal membrane slip and of the amniotic vesicle resulted in increased fetal death. Of interest, significant differences among palpation techniques of individual examiners were found in the detection of fetal loss after examination. Other authors also report that rectal palpation may be a significant iatrogenic cause of fetal death.[17] Still others, however, have been unable to confirm that fetal death after diagnosis of pregnancy is associated with rectal palpation.[18,19] Recently, fetal loss due to palpation of the fetal membrane slip was prospectively measured and compared with fetal loss in heifers diagnosed as pregnant by a noninvasive method.[20] Although substantial loss of embryos occurred between 30 and 60 days after breeding, it was not different between the palpated group and the control group, leading to the conclusion that embryo loss was not caused by rectal palpation.

It is difficult to separate fetal attrition potentially caused by rectal palpation from spontaneous fetal loss in nonpalpated animals. In light of the information currently available, a reasonable conclusion is that if rectal palpation is a cause of fetal death, the incidence probably is low and the value of the information gained is greater than the risk of iatrogenic fetal loss. Nevertheless, clinicians must be aware of the possibility of negative effects of rectal palpation on early pregnancies and conduct examinations meticulously, cautiously, and with dispatch.

Fetal Damage

Palpation of the amniotic vesicle for diagnosis of pregnancy before day 40 has been implicated as a cause of atresia coli in newborn calves.[21,22] Other investigators believe that atresia coli is a genetic defect, and although early-pregnancy palpation may contribute to the deformity, it is not an invariable etiologic factor in all cases of atresia coli.[23] The authors of a retrospective study of 26 cases of this condition found no association between atresia coli and a history of rectal palpation for diagnosis of pregnancy.[24]

Disease Transmission

Bovine leukemia virus (BLV) has been experimentally transmitted to seronegative cattle and sheep by rectal infusion of relatively large amounts of blood from viremic animals.[25] BLV also has been experimentally transmitted to calves by simulated palpation with obstetric sleeves contaminated with whole blood from a seropositive donor animal.[26] Other authors, however, have reported that BLV transmission by rectal palpation in the manner typically used for reproductive tract examination of cows either does not occur or is uncommon.[27] In herds in which other measures to control transmission of BLV are practiced, it may be prudent to use a separate clean obstetric sleeve for palpation of each cow. The role of rectal palpation performed by clinicians wearing common obstetric sleeves in transmission of other infectious diseases is unknown.

Maternal Bacteremia

Bacteria were isolated from blood samples collected from 4 of 35 otherwise healthy cows after rectal palpation of the reproductive organs was performed.[28] The authors of this report concluded that bacteremia following rectal examination of dairy cattle probably was not significant and that no changes in herd health protocols were warranted. However, they pointed out that clinicians should be aware that rectal palpation is not without consequences.

CHEMICAL TESTS FOR PREGNANCY

Progesterone

A number of investigators have attempted to diagnose pregnancy in cows and other domestic animals by measurement of progesterone concentrations in blood or milk samples taken after insemination at about the time of the first expected estrus.[29–31] Progesterone can be measured by laboratories using radioimmunoassays or by various assay kits that are available for on-farm use. This strategy is based on the requirement for luteal progesterone to maintain pregnancy in cows. If a cow is pregnant, she does not return to estrus, and progesterone concentrations in blood and milk are elevated at 20 to 24 days after insemination. Conversely, if a cow has failed to conceive, progesterone concentration is elevated until approximately day 17, when luteolysis is followed by a sharp decline in progesterone concentration by day 20 and return to estrus. Therefore, if progesterone concentrations are low in blood or milk samples taken at 20 to 24 days after insemination, the cow is assumed to be nonpregnant, whereas cows are assumed to be pregnant if progesterone concentrations are elevated.

Measurement of progesterone has been found to be from 75% to 85% accurate in correctly identifying pregnant cows and nearly 100% accurate in identifying nonpregnant cows. In a recently reported field trial, measurement of progesterone in a single milk sample taken on day 21 after insemination had the unacceptably low positive predictive value of 83.0%. If the combination of a low progesterone concentration on the day of insemination and a high progesterone concentration on day 21 after insemination was used, the positive predictive value of the test increased to 87.4%.[32]

False positive results may be due to errors in performing the assay or in sample identification, normal variations in the length of the estrous cycle (shorter than 18 days or longer than 24 days), ovarian abnormalities (luteal cysts), uterine abnormalities (pyometra), and errors in estrus detection that result in insemination during the luteal phase of the estrous cycle. Early embryonic death may lead to apparent false positive results by prolonging the lifespan of the corpus luteum if the embryo is lost after maternal recognition of pregnancy. Elevated milk progesterone concentrations on days 20 to 24 after insemination indicate only the presence of a

corpus luteum, which usually, but not invariably, is associated with a conceptus.

False negative results are unlikely unless due to laboratory error or misidentification of samples. Cows require progesterone to maintain pregnancy, and cows with low concentrations of progesterone are extremely unlikely to be pregnant.

Because progesterone assays are not accurate for identification of pregnant cows, the procedure has not become popular among veterinarians or cattle owners. Measurement of progesterone may be more useful in samples taken on the day of insemination to verify that progesterone concentrations are low and that the cow has not been erroneously identified as being in estrus and presented for insemination during the luteal phase of the estrous cycle.

Estrone Sulfate

Estrone sulfate is a product of the placenta and is present in the milk of pregnant cows in concentrations sufficient to differentiate between pregnant and nonpregnant cows after approximately day 100 of gestation.[32–34] In practice, however, assays for estrone sulfate are not useful for early detection of pregnancy and offer no substantial advantage over other methods for pregnancy diagnosis, except in the few cows in which rectal palpation cannot be performed.

Bovine Pregnancy-Specific Protein B

A pregnancy-specific protein secreted by trophoblastic cells in cows—bovine pregnancy-specific protein (bPSPB)—has been isolated and purified. Radioimmunoassays for measurement of bPSPB have been developed and used to differentiate pregnant from nonpregnant cows.[35–37] Concentrations of bPSPB are detectable in a few cows as early as 15 days after insemination and in nearly all pregnant cows by 24 days after insemination. The protein increases in concentration as gestation advances and is detectable until parturition. In a herd of beef cows, assay for bPSPB was found to detect pregnancy more accurately than rectal palpation. Concentrations of bPSPB are higher in twin pregnancies than in single pregnancies, but high individual variations did not permit accurate prediction of fetal numbers.[38] Assays for bPSPB have been found useful for detection of pregnancy in moose[39] but were not accurate when used in llamas.[40]

Immunosuppressive Early Pregnancy Factor

An assay has been developed for detection of a glycoprotein immunosuppressive early pregnancy factor in the serum of pregnant cows. Blood samples were collected from dairy cows within 24 hours of ovulation and assayed for presence of immunosuppressive early pregnancy factor. The assay was able to diagnose pregnancy in 87.5% of cows at less than 24 hours of gestation and was 12.5% inaccurate in the identification of nonpregnant cows.[41]

ULTRASONOGRAPHY

Real-time ultrasonography has been used for detection of pregnancy in cattle for more than a decade. The principles of ultrasonography and its use in animal reproduction have been described.[42] A number of transducers are available for use with ultrasonographic scanners designed or adapted for use in animals, but 5-MHz and 7.5-MHz transducers are most widely used for transrectal ultrasonography. Lower-frequency transducers are capable of penetrating greater depths of tissue but are not capable of resolving small structures. Higher-frequency transducers are capable of resolving smaller structures but do not penetrate deeply through tissues.

Pregnancy has been diagnosed with a 7.5-MHz transducer as early as 9 days after insemination in heifers.[43] The embryonic vesicle was detected in heifers with a 5-MHz transducer by day 13 or 14 and the embryo observed by days 26 to 29.[44] When a 5-MHz transducer was used to detect pregnancy, accuracy was no better than a guess until day 18 after insemination but then became nearly 100% on days 20 and 22 when examinations were conducted under optimal circumstances.[45] Most clinicians agree that under practical conditions, ultrasonography with a 5-MHz transducer is an accurate method for diagnosis of pregnancy after approximately day 24, when a heartbeat will be detectable in viable embryos. A 3.5-MHz transducer was found to be reliable for detection of pregnancy after day 30.[46]

The appearance of bovine conceptuses when examined with ultrasonography from days 20 through 60 of gestation has been described,[47] and ultrasonographic evaluation of the bovine conceptus has been reviewed.[48] The embryo proper was first detected within the amniotic vesicle on day 20.3, when it was found to be 3.5 mm in length. By day 60, the embryo had grown to 66.1 mm. Between days 28 and 31, forelimb buds became visible, and hindlimb buds were visible approximately 2 days later. Two claws became visible on the hooves between days 42 and 49, and movements of the fetal head and feet were first detected between days 42 and 50. Ribs could be visualized beginning on days 51 to 55. Placentomes were first visualized between days 33 and 38 in the area of the embryo and could be seen throughout the uterine horn by day 60.

The accuracy of ultrasonography for diagnosis of pregnancy may vary among animals. More incorrect diagnoses of nonpregnant were made using a 5-MHz transducer between 24 and 38 days after insemination in cows in which the uterus was located far cranial to the pelvic inlet than in cows in which the uterus was located within or close to the pelvic inlet when examined.[49]

An additional advantage of ultrasonography for diagnosis of pregnancy is the ability to determine fetal gender. Scrotal swellings and teats were detected between days 73 and 120, and gender of the fetus was determined with an accuracy of 94%.[50] Other investigators used ultrasonography to visualize the fetal genital tubercle.[51] On days 48 and 49, the tubercle was located between the hindlimbs, toward the umbilical cord in males and toward the tail in females. In male fetuses, the tubercle was immediately caudal to the umbilical cord by approximately day 56, and in females, the tubercle was under the tail by approximately day 54. Thus, an experienced ultrasonographer was able to accurately determine fetal gender between days 55 and 60.

Ultrasonography is likely to replace rectal palpation as the standard against which other techniques for pregnancy diagnosis are compared. At present, the cost of reliable ultrasonographic scanners precludes their routine use in food animal veterinary practices, but the technique is likely to become more popular as less expensive instruments become available and clients demand the service. Ultrasonographic scanners are reliable and rugged and can be used for examining large numbers of beef and dairy cows if precautions are taken to protect the console and transducer from moisture, dust, and impact. Properly grounded power connections are essential to protect the patient and the operator from electrical shock.

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CHAPTER 40

Ultrasound Determination of Fetal Gender

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Fetal gender determination by real-time ultrasound evaluation has become a common reproductive management tool employed by both dairy and beef cow-calf operations. Other benefits of ultrasonography include early pregnancy diagnosis, diagnosis of fetal viability, twins and uterine pathology, and increased accuracy of ovarian structure identification.[1–3] When fetal gender determination initially was utilized by beef cattle producers, the payback ratio was estimated to be at least 10:1 and sometimes as high as 100:1.[4] It was thought at that time that the high profit margin probably was a result of the newness of the technology and that the procedure would remain profitable even after widespread acceptance. Those predictions have turned out to be accurate, because pregnant beef cows that are marketed with known-gendered fetuses routinely sell for $25 to $50 more per head than contemporaries with unknown fetal gender. Benefits of fetal gender determination for the dairy cow include the increased sale value of pregnant animals carrying heifers, ability to plan embryo transfer programs to fill bull contracts, cull decision information for marginal cows, and ability to predict replacement needs.

To accurately determine fetal gender, it is important that the practitioner have good-quality ultrasound equipment, an excellent understanding of fetal anatomy, and thorough training in reproductive ultrasonography. It also is important for practitioners to recognize the limitations of their ability, and of the technology itself, to ensure producer adoption of fetal gender identification. The reported accuracy of fetal gender determination approaches 100%.[5] Under conditions of low light and calm cattle, this accuracy can be realized. Nevertheless, occasionally, despite the practitioner's best effort, the necessary view cannot be obtained and therefore an accurate diagnosis cannot be made. In such instances it is imperative that the practitioner refrain from guessing; the producer should be informed of the limitations, and the examination be should be repeated in a few days. With experience, nondiagnostic examinations will occur with decreasing frequency.

EQUIPMENT

A number of the ultrasound units available for purchase are more than adequate to identify fetal gender in cattle.

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Ultrasound Determination of Fetal Gender

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Fetal gender determination by real-time ultrasound evaluation has become a common reproductive management tool employed by both dairy and beef cow-calf operations. Other benefits of ultrasonography include early pregnancy diagnosis, diagnosis of fetal viability, twins and uterine pathology, and increased accuracy of ovarian structure identification.[1–3] When fetal gender determination initially was utilized by beef cattle producers, the payback ratio was estimated to be at least 10:1 and sometimes as high as 100:1.[4] It was thought at that time that the high profit margin probably was a result of the newness of the technology and that the procedure would remain profitable even after widespread acceptance. Those predictions have turned out to be accurate, because pregnant beef cows that are marketed with known-gendered fetuses routinely sell for $25 to $50 more per head than contemporaries with unknown fetal gender. Benefits of fetal gender determination for the dairy cow include the increased sale value of pregnant animals carrying heifers, ability to plan embryo transfer programs to fill bull contracts, cull decision information for marginal cows, and ability to predict replacement needs.

To accurately determine fetal gender, it is important that the practitioner have good-quality ultrasound equipment, an excellent understanding of fetal anatomy, and thorough training in reproductive ultrasonography. It also is important for practitioners to recognize the limitations of their ability, and of the technology itself, to ensure producer adoption of fetal gender identification. The reported accuracy of fetal gender determination approaches 100%.[5] Under conditions of low light and calm cattle, this accuracy can be realized. Nevertheless, occasionally, despite the practitioner's best effort, the necessary view cannot be obtained and therefore an accurate diagnosis cannot be made. In such instances it is imperative that the practitioner refrain from guessing; the producer should be informed of the limitations, and the examination be should be repeated in a few days. With experience, nondiagnostic examinations will occur with decreasing frequency.

EQUIPMENT

A number of the ultrasound units available for purchase are more than adequate to identify fetal gender in cattle.

Key areas of consideration in purchasing a scanner are image quality, portability, service, and power source.

Image quality is determined by a complex set of factors. An ultrasound unit has two basic parts, both of which affect image quality: the transducer and the console.

Transducer

The number of elements of piezoelectric crystals in the transducer and the frequency of their vibration influence image quality. The number of crystals (or elements) in the transducer will be greater than 80 in most good machines. The frequency of vibration of the piezoelectric crystals is measured in megahertz (MHz). Transducers for bovine use commonly are available in frequencies of 3.5 MHz, 5 MHz, or 7.5 MHz. Lower-frequency transducers provide more depth of penetration but decreased resolution, so they are unsuitable for reproductive examinations. Higher-frequency transducers provide the best resolution but limit the depth of penetration, which may make scanning larger fetuses difficult. The standard transducer used in bovine reproductive ultrasonography has been the 5-MHz linear transducer, which we prefer in our practice. Some practitioners, however, prefer the increased resolution provided by the 7.5-MHz transducer.

Transducers are available in different shapes: linear, curvilinear, or sector. This designation refers to how the piezoelectric crystals are arranged in the transducer. As the name implies, the linear transducers have the crystals arranged in the head of the transducer in a linear fashion. The curvilinear transducer has a bend in the linear arrangement, and the sector scanner has a single crystal that oscillates, providing a wedge-shaped image. The sector and curvilinear scanners are awkward for routine rectal examinations. At this time, almost all transducers currently available for bovine ultrasonography are linear.

Console

The console should read at least 64 shades of gray. Most units have 256 shades of gray, but the human eye cannot detect anything greater than 64. The number of channels determines focusing ability. Twelve or more channels are adequate. Beyond 30 channels no visible difference will be noted.[6]

Other Components and Considerations

Portability and power source. Ultrasound units for use in the bovine vary in weight from 2 to 35 pounds. The larger units require an outside power source and are designed to be placed on a cart. They are most suitable for situations in which the patient comes to the machine. The smaller portable units are battery operated and are designed to be worn on the person. These are ideal when mobility is required.

Image display. Units may have a screen display, goggles, or both. Screen displays are useful when more than one person wishes to view the scan. Goggles are helpful when working outdoors or in bright light.

Additional options. Fetal aging software, image recording abilities, dual screen display, cine mode, M-mode, and color Doppler are just a few options available on some machines. None are critical for fetal gender diagnosis, but certain options may be desirable in some situations.

Warranty and service. One-year warranties are standard for most ultrasound units. Turnaround time on repairs should be short, perhaps as quick as 48 hours. Some companies will provide a loaner unit during repair.

Choosing an Ultrasound Unit

Image quality is the most critical criterion in selecting an ultrasound unit. A quick way to determine image quality is to scan an ovary with a corpus luteum. The ovarian stroma should be easily differentiated from the luteal tissue. Beyond good image quality, personal preference will determine a practitioner's choice of machine. The practitioner should try as many machine and transducer combinations as possible and then buy the one that feels the most comfortable and that has the best service contract.

ANATOMIC LANDMARKS

The genital tubercle is the prime structure that needs to be identified during an ultrasonographic examination for fetal gender. In the female, the genital tubercle will develop into the clitoris, so it is always located just below the tailhead. The male genital tubercle will develop into the penis, so it is always located immediately behind the umbilical cord. The male and female genital tubercles can be identified as early as day 55 using ultrasound examination.[5] The gestational period during which fetal sexing is most accurate is 60 to 80 days.[7] Before 60 days, the fetus may not be adequately developed; after 80 days, the ultrasound energy may not adequately penetrate maternal tissue to reach the fetus, especially in large dairy cows and overconditioned heifers. Figure 40-1 is a photograph of an 80-day-gestation bovine male fetus, with *arrows* demonstrating the important anatomic landmarks, foot, umbilicus, genital tubercle, and scrotum for fetal gender identification. Figure 40-2 presents a view of the

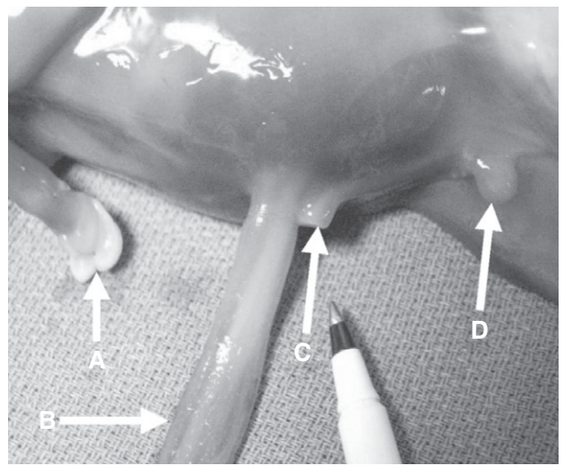

Fig. 40-1 Ultrasound view of the ventral surface of an 80-day bovine male fetus. *A,* Front foot; *B,* umbilicus; *C,* genital tubercle; *D,* scrotum.

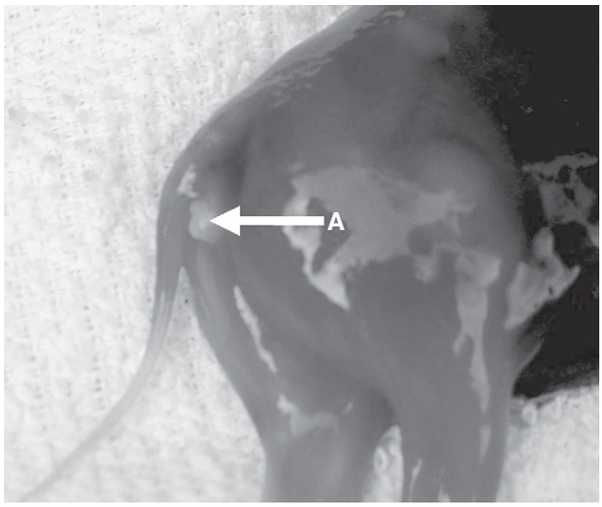

Fig. 40-2 Hindquarters of 75-day female fetus. A female genital tubercle is recognizable.

hindquarters of 75-day-gestation female fetus, with the female genital tubercle identified by the letter A.

Other key landmarks include the head, the caudal-to-cranial taper of the thoracic cavity, the fetal heart, and the fetal stomach and liver. These landmarks give the practitioner a cranial-to-caudal orientation to evaluate which direction the probe should be moved for imaging the genital tubercle. Teats or scrotum also may be seen, especially on fetuses more than 75 days in gestation, but diagnosis of fetal gender should not be based on identification of these structures alone. To have a reasonable chance at becoming proficient at ultrasonographic fetal gender determination, it is critical that the practitioner have an in-depth knowledge of fetal anatomy so that the images received from the ultrasound machine can be accurately interpreted.

GETTING THE IMAGE

After familiarization with fetal anatomy and the critical structures, it is time to make sense of the snowlike images on the screen. Often, the biggest struggle is breaking out of palpation mode, because most practitioners find ultrasound interrogation to be a very different skill from palpation. The transition from tactile to visual diagnosis can be difficult, especially for experienced palpators. In fact, good palpation skills are helpful, but not critical, to develop good ultrasonography skills. Ultrasound is a great teaching tool for novice palpators because they can "see" what they were just feeling. Faith and patience are the two keys in starting to image the reproductive tract of cattle, and in particular when the goal is to identify fetal gender. This section summarizes the basic examination points.

The goal of the examination to determine fetal gender is to identify a gender-specific structure: the genital tubercle. The genital tubercle is composed of highly echogenic (bright white on screen) tissue and usually appears bilobed. In the male, the genital tubercle will be located just caudal to the umbilicus, and in the female, just ventral to the tail. Refer back to Figures 40-1 and 40-2 for orientation. Individual clinicians will develop their own imaging technique as they gain experience. Some prefer to first palpate the uterus and retract it into the pelvic cavity; others, ourselves included, prefer not to palpate and instead immediately scan the uterus. The key is to develop a systematic and efficient approach. The first step is to obtain cranial-to-caudal orientation by visualizing the head, beating heart, and the umbilicus.

Unlike a radiograph, the ultrasound image represents only a thin cross-section of the tissues directly below the transducer. Accordingly, the transducer must be moved to find and identify the location of structures. To center the image on the screen, the transducer must be moved forward and backward. Once the fetus is centered, the transducer can be moved very slowly sideways in both directions to locate the genital tubercle.

Once the ultrasound operator is oriented, one of three basic views can be used to examine the fetus: a **lateral view,** a **frontal view,** and a **cross-sectional view.** Of these basic views, the cross-sectional view, is the most commonly used and easiest view to obtain. If a cross-sectional view is obtained, the transducer should be moved through the fetus to the umbilical attachment in the ventral abdomen and then moved slowly back and forth to diagnose the presence or absence of the male genital tubercle just caudal to the umbilicus. The genital tubercle will always be more echogenic than the umbilicus. If a male genital tubercle is noted, the examination is concluded. If the male genital tubercle is not conclusively identified the transducer should be advanced through the fetus to the perineal region. These simple manipulations sound easy in theory, but in practice the perineal area is relatively difficult to image because it often is obscured by the uterine wall or a placentome. It also is important to be able to distinguish the highly echogenic coccygeal vertebrae of the tail from the genital tubercle. In an oblique view, the pin bones also may be mistaken for the female genital tubercle. In general, the female genital tubercle will be bilobed or trilobed, and the tail or pin bones are monolobed. Another common reason for an incorrect identification of a female as a male is a fetal position in which the bright, echogenic tip of the tail is tucked between the hind legs.[8,9]

The frontal view is a little more difficult to obtain than the cross-sectional view but is the easiest to orient and in our experience provides the clearest view of the genital tubercle and other gender-specific anatomic structures. The ultrasound appearance of the female genital tubercle in the frontal view of the perineal region is demonstrated in Figure 40-3. A similar view of a male fetus is shown in Figure 40-4 demonstrating the scrotum. The lateral view is the least commonly used for fetal gender identification. The male genital tubercle may be obscured by the legs in this view. It also is more difficult to view the female genital tubercle. For these reasons, we rarely use this image.

Key points in the ultrasound examination are summarized in Box 40-1. Table 40-1 presents a sample worksheet for recording the ultrasound findings.

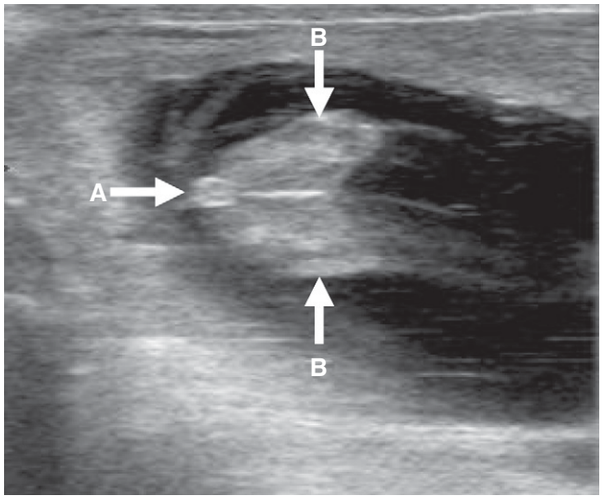

Fig. 40-3 Ultrasound view of the hindquarter of a 75-day female bovine fetus.

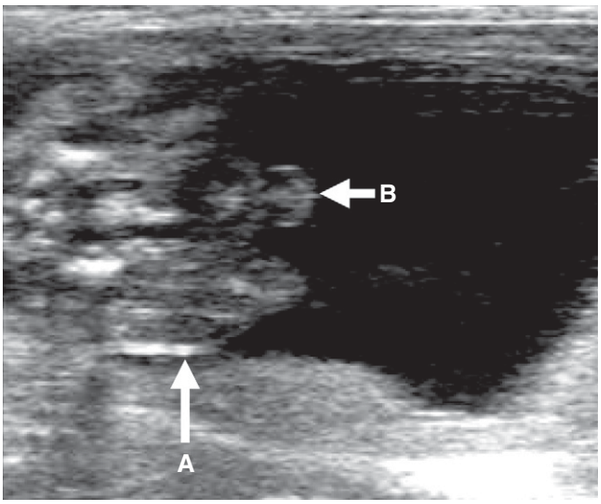

Fig. 40-4 Ultrasound view of the hindquarters of a 80-day male fetus. Image was obtained using a 5-MHz linear probe. The dorsal surface of the fetus is to the *left* in the figure. *A,* Right thigh; *B,* trilobed scrotum.

Table **40-1**

Fetal Age and Sexing Worksheet

Itsa Bullorheifer, DVM
Ultrasound Services, Inc./LLC
123-555-6789
Date: _____
Client: _____

ID	Date Bred or Fetal Age	Predicted Sex	Expected Calving Date	Comments

Research indicates that fetal sexing is 95–99% accurate.
Because of variability in fetal size and gestation length between animals, calving dates may deviate up to 1 month from fetal age measurements.
Although no research indicates that ultrasound is harmful to the fetus, no warranty is made for fetal viability beyond the date of examination.
No warranty is made on fetal sex determination or fetal aging.
Adapted from Colloton JD: American Association of Bovine Practitioners Seminar on Bovine Ultrasonography Notes. Vancouver, BC, Canada, September 2001.

SUMMARY

Achieving technical competence in ultrasonographic fetal gender determination will require (1) a high-quality ultrasound unit, (2) a detailed understanding and comprehension of ultrasonographic fetal anatomy, and (3) the skill to manipulate the ultrasound transducer to get a well-positioned, adequately focused image. The first two of these requirements are relatively easy to achieve; the third requires practice and patience. It is important to make the investment in training both through video tapes and in participation in "wet labs" and seminars. With commitment and the requisite investment, professional competence can be achieved.

Box 40-1

Fetal Sexing by Ultrasonographic Evaluation: Key Points

1. Fetal age: 60 to 80 days of gestation. The ultrasound examination can be performed as early as day 55 and as late as day 110.
2. Diagnosis is based only on the identification of the genital tubercle or gender-specific structure such as the penis.
3. Move the transducer in a slow, controlled fashion.
4. Keep your eye on the screen all the time, because sometimes structures move by quickly.
5. Locate the umbilicus and move caudally.
6. If possible, roll the transducer under the uterus to provide the best contact between the transducer and the fetus, for optimal focus and clarity.
7. Check for twins during the examination.
8. Keep careful records of the findings (see Table 40-1).
9. Stay relaxed, and focus on making smooth, controlled movements with the transducer.

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1. Beal WE, Perry RC, Corah LR: The use of ultrasound in monitoring reproductive physiology of beef cattle. *J Anim Sci* 1992;70:924–929.
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CHAPTER 41

Induced Abortion

PHILIP G. A. THOMAS

Therapeutic abortion may be indicated during normal or abnormal pregnancy in the cow. Misidentification of a breeding female, accidental breeding of a very young heifer, and unwanted pregnancy in feedlot heifers are indications for abortion during normal gestation. Induced abortion can be included in treatment protocols for pathologic conditions of pregnancy, including fetal maceration, fetal mummification, hydramnios, and hydroallantois.

PHYSIOLOGY OF PREGNANCY MAINTENANCE

Gestation in the cow extends 270 to 292 days after breeding. Once conception has occurred, progesterone is essential for pregnancy maintenance. Both luteal and extraovarian sources of progesterone must be eliminated for successful induction of abortion. Although the maternal endocrine events of the first 15 days of cycle and of pregnancy are similar, the conceptus secretes a range of products, including steroids, prostaglandins, and proteins, beginning at 12 to 13 days of gestation. At least one of these products, interferon-γ, results in maternal recognition of pregnancy by inhibition of luteolysis and prolonged luteal lifespan.[1–3] These effects are mediated by attenuation of endogenous prostaglandin F_2 alpha ($PGF_{2\alpha}$) secretion.[4]

The functional life of the corpus luteum (CL) is controlled by a balance of luteotropic factors, including luteinizing hormone, and luteolytic factors, including $PGF_{2\alpha}$. $PGF_{2\alpha}$ is the naturally occurring luteolysin, acting both directly and indirectly on the CL. $PGF_{2\alpha}$ may cause local vasoconstriction of luteal blood flow; however, $PGF_{2\alpha}$ receptors are present on luteal cells, and $PGF_{2\alpha}$ has a direct effect on luteal progesterone secretion.[5] Endogenous luteolysis occurs in response to a cascade of hormonal events that result in pulsatile $PGF_{2\alpha}$ secretion. It has been proposed that as part of this cascade, estradiol

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induces oxytocin receptors on endometrial cells.[6] Oxytocin activates those receptors, resulting in the synthesis and secretion of $PGF_{2\alpha}$.

Peripheral progesterone levels fluctuate between 6 and 15 ng/mL throughout gestation,[7] with a gradual decline in the 2 to 4 weeks preceding parturition.[8] Pregnant cows that undergo ovariectomy before the first 3 to 5 months of gestation will abort. In cows ovariectomized after 200 days of gestation, progesterone secretion is maintained, although at significantly lower levels than before ovariectomy.[9] These cows do not abort, but parturition is advanced by 2 weeks and may be accompanied by dystocia and retained fetal membranes. The adrenal gland may contribute 1 to 4 ng/mL of progesterone.[10]

In summary, progesterone is luteal in origin for the first 150 days of gestation. Between 150 and 250 days, the placenta acts as additional source of progesterone. In the final month of gestation, placental progesterone declines and pregnancy is again dependent on luteal progesterone. Successful treatment to induce abortion must lower circulating progesterone below 1 ng/mL, which is the threshold necessary to maintain pregnancy,[11] and must be directed specifically at the source of progesterone appropriate for the stage of gestation at the time of treatment.

MECHANISM OF ACTION OF THERAPEUTIC AGENTS

Prostaglandin F_2 Alpha

The CL is sensitive to $PGF_{2\alpha}$ beginning 5 to 7 days after ovulation. In both normal and abnormal pregnancy, administration of $PGF_{2\alpha}$ after that time results in luteolysis at any stage of pregnancy; however, $PGF_{2\alpha}$ treatment alone induces abortion only up to 5 months of gestation. Rarely, luteolysis is incomplete, in which case luteal progesterone remains above the threshold, and partial cervical dilation and abdominal straining may occur before the cow resumes normal gestation.[11]

Glucocorticoids

Glucocorticoid treatment appears to reduce placental progesterone secretion from 150 days of gestation. Luteal progesterone is unaffected, however, and abortion does not result from glucocorticoid treatment until the last month of gestation. During the final month of gestation, glucocorticoids act at the fetoplacental unit to increase the production of estradiol and $PGF_{2\alpha}$, resulting in induced parturition. A combination of $PGF_{2\alpha}$ and glucocorticoids will induce abortion from 150 days of gestation.

Estrogens

Treatment with estrogens during the first 2 to 3 days after ovulation alters oviductal transport of the bovine embryo and terminates pregnancy. After the development of the CL, estrogens cause luteolysis by inducing the endogenous $PGF_{2\alpha}$ luteolytic cascade from the endometrium, as described earlier. The endometrium must be intact for estrogens to induce abortion. Estrogen is an exogenous luteolysin with unknown effects on the fetoplacental unit; therefore, abortion can be induced reliably at up to 150 days of gestation. Treatment of cows between 200 and 220 days of gestation with 30 mg estradiol valerate, alone or in combination with dexamethasone, has not been shown to decrease serum progesterone or result in abortion, however.[11]

Treatment with estradiol or its synthetic derivatives results in prolonged estrous behavior, vulvar swelling, mucopurulent discharge, and relaxation of parts of the posterior reproductive tract. The function of the uterotubal junction as a sphincter may be impaired, possibly allowing ascending infection and salpingitis. Time to return to fertile estrus after estrogen treatment may be longer than after prostaglandin treatment.

Oxytocin

Treatment of cows with oxytocin from days 2 to 7 after estrus with 100 to 200 IU of oxytocin prevents pregnancy, probably by preventing normal luteal development.

TREATMENT STRATEGIES IN TERMINATING PREGNANCY

Normal Pregnancy up to 150 Days

In the first 5 months of pregnancy, the treatment of choice is $PGF_{2\alpha}$ or an analogue (Table 41-1). Cows do not respond until 5 to 7 days after ovulation. After that time, a majority of cows respond by returning to fertile estrus within 3 to 5 days of treatment.[12,13] Cows not in estrus within 5 days should be retreated. Treatment with oxytocin in the first few days after ovulation may prevent the establishment of pregnancy. Between days 5 and 10 after ovulation, intrauterine infusion of irritating solutions

Table **41-1**

Drugs and Doses Commonly Used for Induction of Abortion during Normal Pregnancy in the Cow

Drug(s)	Dose and Route	Days to Onset of Effect
Prostaglandin $F_{2\alpha}$	25 mg IM	5–150
Cloprostenol	500 µg IM	5–150
Fenprostalene	1 mg SC	5–150
Dexamethasone	25 mg IM	275–283
Dexamethasone plus prostaglandin $F_{2\alpha}$[†]	25 mg 25 mg IM	5–283
Diethylstilbestrol	40–80 mg IM	1–3
	150 mg IM	5–150
Estradiol esters[‡]	4–8 mg IM	1–3
	10–20 mg IM	5–150

*Days after ovulation.
[†]Other combinations include dexamethasone (25 mg) plus cloprostenol (500 µg) and dexamethasone (10 mg) plus cloprostenol (375 µg).
[‡]Esters of estradiol include estradiol valerate, estradiol benzoate, and estradiol cypionate.
IM, intramuscularly; SC, subcutaneously.

prevents the establishment of pregnancy and may cause luteolysis and early return to estrus. Infusion later than 11 days after ovulation occasionally lengthens the estrous cycle. Up to 90 days of gestation, the same strategy causes embryonic death necessitating manual evacuation of uterine contents. Suitable solutions include 0.5% percent aqueous iodine and 2 g tetracycline in saline.

Estrogens administered within 72 hours of ovulation impede oviductal transit of embryos. Up to 5 months of gestation, administration of an estradiol ester, such as estradiol valerate, results in abortion within 7 days (see Table 41-1). Occasional abortions occur up to 14 days after treatment. Estradiol should be administered every 4 days until abortion. Abortion was reported in 60% to 80% of heifers within 7 days of a single treatment.[11]

Manual techniques are of historical interest only. Manual enucleation of the CL by transrectal manipulation removes progesterone support for pregnancy and results in abortion at up to 150 days, but this technique may induce adhesions of the ovary and ovarian bursa and, occasionally, severe hemorrhage.[14] Manual rupture of the amniotic vesicle by transrectal manipulation is possible once the vesicle can be palpated at 30 to 35 days of gestation. After 60 days and up to 120 days of gestation, when the amniotic vesicle can no longer be isolated within the fluid-filled chorioallantois, it may be possible to terminate pregnancy by manual decapitation of the fetus. The mean time to abortion is 25 days, but abortion may occur up to 8 weeks after treatment.

Normal Pregnancy after 150 Days

Between 5 and 8 months of gestation, a combination of $PGF_{2\alpha}$ and dexamethasone is necessary to remove both luteal and extraovarian sources of progesterone. This combination is preferable to all other treatments. Abortion will occur reliably, with a mean time to abortion of 5 days.[11] Abortion may be preceded or accompanied by estrous behavior for a duration of 9 to 12 hours. This combination is approximately 95% effective, although repeat treatments occasionally are necessary.

Unwanted pregnancy in feedlot heifers results in costs associated with reduced feed conversion, lower carcass prices, and periparturient diseases including dystocia. Heifers should be examined on arrival at the feedlot to select appropriate candidates. All pregnant feedlot heifers are routinely treated with the combination of $PGF_{2\alpha}$ and dexamethasone, regardless of their stage of gestation. Progestin-containing growth promotants should not be used until after abortion has been induced, because these compounds may interfere with treatment. All heifers should be examined after treatment. Those still pregnant usually will respond to a second treatment.

In the final month of pregnancy, either dexamethasone or prostaglandin alone induces premature parturition within 2 to 3 days.

SEQUELS TO INDUCED ABORTION

Induced abortion after the fourth month of gestation results in an approximately 80% incidence of retained fetal membranes. In a majority of cases, fetal membranes are lost within 7 days without treatment. Fetal mummification develops in 2% to 4% of pregnant feedlot heifers treated with a combination of $PGF_{2\alpha}$ and dexamethasone. In occasional cows, metritis or pyometra will develop after induced abortion; however, acute toxic metritis is an unusual sequel.

Hydroallantois and Hydramnios

The pathophysiology and treatment of hydropic conditions of the uterus is addressed elsewhere in this book. Slaughter is recommended for most cows with hydroallantois. Pregnancy can be terminated within 48 hours in cows with hydramnios or hydroallantois with simultaneous administration of prostaglandin and dexamethasone, using doses recommended for normal pregnancy[11,13,14]; however, supportive treatment is necessary to compensate for lost fluid, and parturition usually is abnormal. Cesarean section may be an alternative to induced parturition.

Fetal Mummification and Maceration

Fetal death without luteolysis of the maternal CL results in fetal mummification or maceration. **Maceration** can occur at any stage of gestation, although usually it is noted only after fetal bone calcification has commenced. Maceration is accompanied by a partially dilated cervix and possibly a fetid discharge apparent in the vagina. **Mummification** becomes evident between 3 and 8 months of gestation and is characterized by autolysis and fluid reabsorption in a sterile environment.[14]

Prostaglandin $F_{2\alpha}$ or an analogue is the therapeutic agent of choice for fetal mummification, with excellent prognosis for return to fertility within 1 to 3 months. Expulsion of the fetus usually occurs within 24 to 72 hours. Retreatment of cows with mummified fetuses still present at reexamination occasionally is necessary. Luteolytic doses of estrogen (see Table 41-1) also results in expulsion of mummified fetuses. Repeated treatments may be necessary at 48-hour intervals.[10,13–15] After treatment, the mummified fetus may become lodged in the vagina, requiring lubrication and manual removal.

Fetal maceration usually results in chronic maternal endometrial damage; therefore, slaughter may be recommended. Response to treatment with $PGF_{2\alpha}$ or estrogen is unrewarding. Macerated bones may be removed at surgery or through a partially dilated cervix, before or after administration of $PGF_{2\alpha}$ or estradiol[14]; however, endometrial damage carries a poor prognosis for return to fertility. Glucocorticoids are ineffective in treating fetal maceration and mummification, because an intact fetoplacental unit is necessary for their mode of action.

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CHAPTER 42

Parturition and Dystocia

SCOTT NORMAN and ROBERT S. YOUNGQUIST

Dystocia (difficult birth) occurs when the first or second stage of labor is prolonged and assistance is required for delivery. No clear boundaries exist between dystocia and eutocia (normal birth), but guidelines based on progress and duration of the delivery may aid the veterinarian and the producer in deciding when to interfere with the birth process. In the last century, a lot of improvement occurred in the techniques used to deliver and resuscitate calves. Only in the last three decades, however, has research been effectively directed toward the causes and management of dystocia. This may be associated with the more widespread implementation of herd health programs and the use of computers to facilitate data management. The incidence of dystocia varies but generally is more common among first-calf heifers, because they have not yet reached their mature size, and then decreases with age.[1] Death due to dystocia or as a result of injuries sustained during delivery is the most common cause of calf loss during the first 96 hours post partum, with most losses occurring during the first 24 hours after delivery.[2,3] The subsequent pregnancy rate among dams that suffer dystocia also is reduced.[4–6] Although it is not possible to eliminate dystocia, improvements in management of heifers during their development and observation of cows and heifers during the calving season are critical for reducing calf losses.

This chapter begins with a description of the process of normal parturition (eutocia). The diagnosis and treatment of dystocia are described next. Finally, owing to the importance of dystocia control and prevention, management techniques used to reduce the incidence of dystocia are discussed, with the main focus on specific control measures.

PARTURITION

During the last weeks of gestation, the dam prepares for delivery of the fetus and the initiation of lactation. Enlargement of the udder may begin in heifers at 5 to 6

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This chapter begins with a description of the process of normal parturition (eutocia). The diagnosis and treatment of dystocia are described next. Finally, owing to the importance of dystocia control and prevention, management techniques used to reduce the incidence of dystocia are discussed, with the main focus on specific control measures.

PARTURITION

During the last weeks of gestation, the dam prepares for delivery of the fetus and the initiation of lactation. Enlargement of the udder may begin in heifers at 5 to 6

months of gestation but may not be obvious in pluriparous cows until the last weeks of pregnancy. The gland may become tightly swollen, and in severe cases, edema may be so extensive in dairy cows as to require induction of parturition or initiation of milking several days before calving. Initial secretions that can be expressed from the mammary gland are viscid and pale yellow to amber in color. As parturition approaches, colostrum is secreted and is white to yellow, turbid, and opaque. The pelvic ligaments relax under the influence of estrogens, relaxin, and the general hormonal milieu that initiates parturition; the gluteal muscles sink, the tailhead becomes more prominent, and the cranial border of the sacrosciatic ligament becomes less tense. A few hours before calving, the vulva becomes edematous, and the cleft elongates. Unfortunately, none of the signs of approaching parturition are specific enough to permit a precise prediction of the exact time of calving; thus, veterinarians have been admonished "to refrain from making too positive or definite a statement regarding the exact time of parturition, as subsequent events will more often than not prove him wrong."[7] Once initiated, delivery of a calf is a continuous process, but for the convenience of discussion, most authors divide labor into three stages or phases.

The **first stage of labor** signals the beginning of delivery and is characterized by progressive relaxation and dilation of the cervix.[8,9] Increased output of adrenocorticotropic hormone (ACTH) and associated cortisol from the fetal adrenals leads to an increase in the level of the enzyme 17α-hydroxylase in the fetal placenta.[10] This in turn reduces placental progesterone production by allowing pregnenolone to be metabolized through to dehydroepiandrosterone. Maternal plasma progesterone concentrations then decrease because pregnenolone is diverted through to estrogen production, and maternal plasma estrogen concentrations subsequently increase. In addition to converting progesterone to estradiol, fetal corticoids also cause the placenta to synthesize prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The synthesis of $PGF_{2\alpha}$ helps abolish the progesterone block to myometrial contractility. As both estradiol and prostaglandin become elevated, the myometrium becomes increasingly more motile and begins to display noticeable contractions. Also, $PGF_{2\alpha}$ causes the corpus luteum of pregnancy to regress, facilitating the decline in progesterone.

As the pressure inside the uterus continues to increase, the fetus rotates so that its presentation is with the front feet and head pointing toward the caudal aspect of the dam. This rotation is important to ensure an uninterrupted delivery. If the fetus fails to achieve the correct presentation, position, or posture, because of either disease or lack of space, dystocia may occur.

The external cervical os of cows may be sufficiently relaxed to admit two to four fingers as much as a week before calving, but the cervix of heifers usually remains tightly closed until the day before calving, when it begins to relax. Dilation of the cervix occurs in two phases. During the preliminary phase, the cervix dilates passively by a decrease in cervical tone. Dilation of the internal cervical os begins 2 to 4 hours after the external os reaches a diameter of 6 to 12 cm. The active phase of cervical dilatation begins approximately when the external os has dilated sufficiently to permit the introduction of a hand and is initiated by increased concentrations of estradiol, coupled with the elevation in levels of $PGF_{2\alpha}$. These hormones induce strong contractions of the myometrium that pull the cervix open and force the fetus and its membranes into the partially dilated cervical canal. Complete cervical effacement occurs when a portion of the fetus enters the cervix and applies mechanical pressure from within the canal.

Pressure on the cervix brought about by increased myometrial contractions and presence of the fetus activates pressure-sensitive neurons located in the cervix that synapse in the spinal cord and eventually synapse with oxytocin-producing neurons in the hypothalamus. Oxytocin released into the systemic circulation acts to facilitate the myometrial contractility initiated by estradiol and $PGF_{2\alpha}$. As pressure against a cervix continues to increase, so does the oxytocin secretion, and the force of contraction of the myometrial smooth muscle begins to peak. With application of this maximal force, the fetal head and forelegs completely enter the cervical canal, the chorioallantois usually ruptures, and the first stage of parturition is complete. At the end of the first stage of labor, the cervix and the vagina are a continuous canal.

It occasionally may be necessary to differentiate between dystocia caused by incomplete cervical dilatation and prolonged first-stage labor. A differential feature of the conditions is that progressive enlargement of the cervical canal can be discerned between two consecutive examinations in cases of prolonged first-stage labor, whereas the status of the cervix remains unchanged in cases of incomplete dilatation.

Clinical signs associated with the first stage of labor most often are observed in primiparous animals; the signs may be minimal or pass unnoticed in older dams. The signs of the first stage of labor generally are those associated with abdominal discomfort and include a variable degree of anorexia, restlessness, shifts of weight from one leg to another, and presence of an arched back with elevated tail. If given the opportunity, most dams will seek solitude away from the remainder of the herd. Some dams may demonstrate mild and intermittent abdominal straining during the late portions of the first stage; thus, the demarcation between the first and the second stage is not as clear in cows as it is in mares. Rupture of the chorioallantois and release of the allantoic fluid ("breaking water") may be a more accurate attribute by which to mark the end of the first stage of labor. The average duration of the first stage of labor is approximately 6 hours, but considerable variation among animals is observed, and the stage may last up to 24 hours in heifers.[11]

The fetus is delivered during the **second stage of labor**, which is characterized by the application of strong abdominal pressure by the dam. Myometrial contractions stimulated by oxytocin force the fetus into and stretch the cervical canal. In particular, the conical shape of the head as the nose enters the cervical canal is important because it progressively dilates the cervix with mechanical pressure. Stretching stimulates the release of more oxytocin from the maternal pituitary gland, which causes further uterine contractions. Oxytocin is thought also

to stimulate release of prostaglandins from the endometrium, which further stimulates myometrial contractions.[12] This positive feed-forward system makes it difficult to stop labor once it has begun. Forms of dystocia that delay or prevent entry of the head or limbs of the fetus into the cervix, to stimulate the pressure receptors, result in little if any abdominal straining by the cow. A typical example is cranial presentation with full flexion of the hips (true breech presentation).

The intact amnion should appear at the vulva as a fluid-filled sac shortly after rupture of the chorioallantois. After the amnion appears, the dam alternates frequently between standing and recumbency. The amnion usually ruptures while the dam is recumbent, and rupture is followed by regular bouts of abdominal straining. Early in the second stage of labor, each bout of abdominal straining consists of 5 to 7 abdominal contractions, which increases to 8 to 10 contractions as the process advances. The cow usually rests in sternal recumbency and may eat between bouts of abdominal straining. As delivery continues, the length of rest periods decreases and the force of abdominal contractions increases. As the contractions become more forceful, the dam rolls to lateral recumbency and lifts the head, hindquarters, and uppermost limbs and may vocalize with each effort. Maximal force is required to deliver the fetal head through the vulva, and in most instances of eutocia the remainder of the fetal body follows with little or no additional effort. In the case of large calves, additional abdominal pressure may be required to deliver the fetal shoulders or hindquarters. Most cows deliver their calves while in lateral recumbency, but a few may complete delivery while standing. Delivery while standing is more common in pluriparous dams than in heifers and may be the result of unintentional disturbance of the cow caused by handlers.

The average length of the second stage of labor in pluriparous cows is 2 to 4 hours, but this stage is longer in heifers because more effort is required to dilate the tissues of the birth canal. A healthy bovine fetus can survive for up to 8 hours after the beginning of the second stage of labor provided that the umbilicus remains intact; survival is longer when uterine contractions are weak or infrequent. Exhaustion and secondary uterine inertia result in cessation of uterine and abdominal contractions after 8 to 12 hours of labor.

The **third stage of labor** is characterized by detachment and expulsion of the cotyledonary placenta. Expulsion of the fetal membranes requires that the chorionic villi become dislodged from the crypts of the maternal side of the placenta. This release of the chorionic villi is believed to be brought about by powerful vasoconstriction of arteries in the villi associated with continued myometrial contractions. Uterine contractions continue after delivery of the fetus and sometimes are fortified by occasional bouts of abdominal straining. Myometrial contractions gradually subside in frequency and amplitude and are not detectable by 2 to 4 days after calving. The time required for expulsion of the placenta averages 8 hours but can range from a few minutes up to 12 hours without being considered abnormal. Most dams rise during the third stage of labor and begin to groom their calf, licking first its hindquarters and then the head and neck. Dams usually continue to face and lick their calves after they stand but soon allow the calf to move alongside, seek the udder, and nurse.

FACTORS CAUSING DYSTOCIA

Causes of dystocia related to management practices are discussed later in the chapter. From a clinical perspective, the etiology of dystocia is multifaceted and includes defects in the dam or the fetus and management factors, or a combination.[13] For purposes of formulating a clinical management plan for an individual animal, it is convenient to divide the causes of dystocia into those of maternal origin and those of fetal origin. It is important to remember, however, that clinical cases may result from multiple underlying disorders and also may require medical treatment to correct concurrent problems, in addition to the more traditional mechanical or surgical treatments.

Maternal Causes of Dystocia

Problems with the dam that impede or prevent delivery include a lack of expulsive force and abnormalities of the birth canal.

Primary uterine inertia. Primary uterine inertia is characterized by failure of the myometrium to contract normally and bring the fetus into the cervical canal. The condition is encountered occasionally in cows, and causes that have been suggested include overstretching of the uterus by multiple or abnormal fetuses, a defect in the myometrium that renders it unable to contract normally, a defect in the hormonal milieu (see the description relating to ineffective labor and physiologic dystocia under "Management and Prevention of Dystocia" later on), and periparturient hypocalcemia. The dam may exhibit a few weak abdominal contractions but does not progress to the second stage of labor. On examination, the cervix is found to be dilated but the fetus has not yet entered the birth canal. The fetal membranes may be intact if labor has not been prolonged. Calves usually are delivered by gentle traction after correction of any defects in posture or position.

Secondary uterine inertia. Secondary uterine inertia is a result of exhaustion of the myometrium after prolonged unsuccessful attempts to deliver a fetus. Treatment is directed at removing the impediment and delivering the fetus by a method appropriate for the clinical circumstances. Sequelae of secondary uterine inertia include retained placenta, delayed uterine involution, and uterine prolapse.

Abnormalities of the birth canal. Delivery may be inhibited by inadequate size of the maternal pelvis, pelvic deformities or exostoses, incomplete dilatation of the cervix, vaginal cystocele, neoplasms of the vulva and vagina, remnants of the müllerian ducts persisting as bands of tissue from the dorsal to ventral walls of the vagina immediately caudal to the cervix, and uterine torsion. Stenosis of the vulva and vestibule may be the result of immaturity or may be a heritable defect in some breeds.

Fetal Causes of Dystocia

Broadly, the fetal origins of dystocia in cattle can be divided into those caused by abnormalities of the fetus (defects in fetal disposition and various forms of maldevelopment resulting in fetal monsters) and those caused by excessive fetal size relative to the maternal pelvis (fetopelvic disproportion).

Abnormal fetal presentation, position, and posture. See the later section "Mutation of Abnormal Presentation, Position, and Posture" for definitions of these and related terms. For normal delivery in cattle, the fetus is in cranial longitudinal presentation and in dorsosacral position, with the head, neck, and forelimbs extended. Caudal presentations are considered abnormal in cattle, but unassisted delivery can occur with the fetus presented caudally if the hindlimbs are extended. Spontaneous delivery with other fetal presentations, positions, or postures is unlikely unless the fetus is quite small or the dam's pelvis is unusually large.

Fetal monsters. A variety of malformations resulting in specific fetal phenotypes and conjoined twins have been described as sporadic causes of dystocia in cattle. Among the fetal monsters more likely to be encountered in cattle are schistosomia reflexus and perosomus elumbus. **Schistosomia reflexus**, shown in Figure 42-1, is characterized by extreme ventral curvature of the spine, so that the head is positioned near the sacrum. The abdominal and thoracic walls are not closed, and the viscera are exposed. Limbs of the affected fetus frequently are rigid because of ankylosis of the joints.

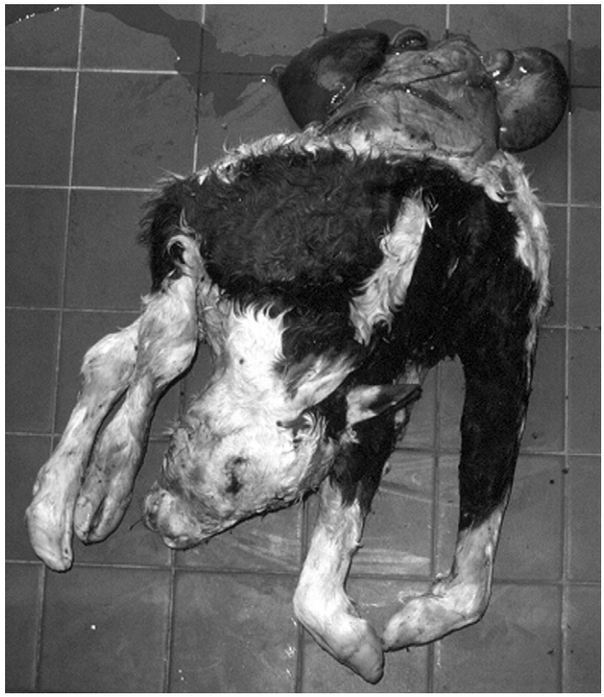

Fig. 42-1 Schistosomia reflexus. These fetuses often are presented in dorsal or ventral transverse presentation. Delivery can be by fetotomy or cesarean section.

Perosomus elumbus is characterized by a nearly normal fetal forepart but flexure and ankylosis of the hindlimbs. Vertebrae are absent caudal to the thorax, and the pelvis is deformed and flattened.

Fetal oversize. The most common cause of dystocia in cattle is fetopelvic disproportion. The situation is most common in heifers where the fetus is of normal size for its breed but the maternal pelvis is of insufficient size (relative oversize) or the fetus may be unusually large and cannot be delivered through a pelvic canal of normal size.

CASE MANAGEMENT OF DYSTOCIA

The spectrum of clinical presentations encountered by veterinary obstetricians is immense. The diagnosis of dystocia usually is made by the owner or manager of the animal, who may decide to seek professional assistance early in the course of delivery or not until labor has been unduly prolonged.

Review of History

The first step in clinical evaluation of bovine dystocia is to obtain as much pertinent history as possible. Although the usefulness and accuracy of the history will vary with the diligence of the manager, the clinician should attempt to obtain at least a minimal amount of information before initiating the examination.

Previous occurrences. If the clinician is not already familiar with the client's management style, it can be useful to gain a brief summary of previous dystocia cases and breeder management. Information on the bull used for the mating period also may be useful in determining the etiology of the current problem, in addition to providing insight for future recommendations. To save time, much of this information can be extracted during conversation as the clinical evaluation proceeds.

Gestation length. The breeding date, if known, should be ascertained. If delivery is premature and dystocia is associated with abortion, the clinician can take steps to reduce or eliminate exposure to infectious causes of bovine abortion that are zoonotic. Prolonged gestation is in most cases associated with excessive fetal size and poor viability.

Progress of the case. It is important to know how long the animal has been in labor. Cows and heifers should be allowed a reasonable amount of time to spontaneously deliver their calves. It is common for cows to vocalize during straining early in stage two of parturition. This is associated with stretching of the soft tissues of the birth canal.[14] Thus, although clinical judgment is necessary, the amount of vocalization during straining may be used as a rough guide to the duration of stage two if the history is not available. If an adequate time for the first or second stage of labor has been exceeded, examination is indicated. Heifers should be allowed a longer time for spontaneous delivery than is required in pluriparous cows. If intervals between observations are excessive (more than 3 hours), the manager will be unable to accurately determine the time of onset of labor, and immediate intervention may be justified. Early obstetric assistance has

been shown to improve the subsequent fertility of beef cows.[15]

The character of the expulsive efforts should be determined—whether they are weak and sporadic or substantial and frequent. In some individual animals, stage two labor may be manifested as only a few weak attempts to deliver the fetus before secondary uterine inertia supervenes. Vigorous and forceful attempts should result in steady progress; if they do not, the dam should be examined.

The clinician should determine if the fetal membranes have ruptured. Rupture of the chorioallantoic membrane and release of the watery allantoic fluid serve to indicate the onset of the second stage of labor. Rupture of the amnion is followed by release of the viscous amniotic fluid. Examination is indicated if delivery is not complete within 2 hours after the amnion appears outside the vulva.

It is important to determine if the patient has been examined, or if any attempts have been made to deliver the calf before attendants sought professional assistance. The ability of managers to determine the cause of dystocia varies, but the results of a proficient examination may be useful to the clinician. Information regarding futile attempts at delivery by attendants may be difficult to elicit, but damage to the birth canal or fetus caused by well-intentioned but inappropriate efforts should be discovered before intervention lest the veterinarian be held responsible.

Restraint

Accessible facilities and demeanor of the patient often dictate the restraint used for examination and relief of dystocia. Facilities and conditions often are less than optimal. Ideally, however, confinement of the parturient dam should be in a dry, well-bedded enclosure of generous size and with sufficient illumination. Difficult vaginal deliveries often are assisted if the dam can be placed in lateral recumbency; therefore, room to cast and restrain a recumbent animal is ideal. Access to a clean water supply also is desirable. A squeeze chute may be suitable for the initial examination, but the inclination of most cows to become recumbent during traction makes this choice undesirable in all but the simplest of deliveries. Although extraction of the fetus is better performed with the dam in lateral recumbency, the initial examination is more readily performed with the animal standing, because pressure within the abdomen is reduced in this posture. Thus, the patient is most conveniently restrained with a halter, but a stanchion or head bail may be used if caution is exercised to prevent asphyxiation or excessive pressure on the carotid arteries should the animal become recumbent during the examination. During delivery, sufficient space should be available behind the patient to allow manipulation of a fetal extractor or fetatome. A position of lateral recumbency can be achieved with a combination of ropes and sedation. Figure 42-2 shows ropes set on a heifer in preparation for casting in lateral recumbency once diagnostic traction has confirmed that vaginal delivery is possible.

Fig. 42-2 Rope placement for casting a heifer before vaginal delivery of the fetus. The side of the chute has been opened in preparation for releasing the head bail and allowing the heifer to exit through the side of the chute. Note that the halter rope is pulled back through the head bail and tied down low to the ground. Sedation may be administered immediately before opening the chute if necessary.

Administration of an epidural anesthetic is not routinely performed if vaginal delivery is to be used, because in most instances the expulsive efforts of the dam are beneficial in assisting delivery. Desensitization of the birth canal and the perineal area usually is reserved for cases that require moderate to extensive manipulation of the fetus before extraction, fetotomy, or surgical delivery is attempted.

Examination

Physical Examination

The clinician should note the general condition of the patient and identify abnormalities that may potentially influence the selection of a method to relieve the dystocia or have an impact on the prognosis. For example, overconditioned feedlot heifers have excessive deposits of fat in the pelvic canal, which reduces its caliber and increases the difficulty of delivery. Recumbent animals should be examined for the possibility of exhaustion, calving paralysis, or hypocalcemia. Pallor of the mucous membranes may suggest internal hemorrhage due to rupture of one of the large blood vessels that supply the uterus. The character of any discharge from the birth canal or any exposed portion of the fetus or membranes should be noted. Fetid or sanguineous discharges are more typical of protracted cases. Yellow-brown discoloration of the fetus or fluids by meconium is an indication of fetal hypoxia, and immediate intervention is indicated.

Rectal Examination

Examination of the reproductive organs by palpation per rectum is indicated in only a few cases of dystocia. The most common indication for rectal palpation is to confirm uterine torsion when stenosis of the cranial vagina is detected during a vaginal examination. Pelvic deformities and exostoses may be more readily detected by palpation per rectum than by vaginal examination.

Other indications for palpation per rectum include elicitation of spontaneous movements of the fetus when it is not possible by vaginal examination, confirmation of uterine rupture, and recognition of hemorrhage into the broad ligaments. Although unusual in cattle, firm feces should be removed from the rectum before the application of extractive force.

Vaginal Examination

Vaginal examination in dystocia almost always implies manual entry into the birth canal. In rare cases, however, speculum examination may be more suitable than manual techniques. Such cases include those in which straining is minimal and conception dates are only approximate.

Two essential requirements for effective obstetric intervention with minimal post-treatment complications are cleanliness and lubrication. Before invasion of the birth canal, the vulva and perineal area and any protruding fetal parts are washed with surgical soap and water. The hands and arms of the operator also are cleansed. Some clinicians prefer to wear shoulder-length plastic sleeves, whereas others find that wearing these sleeves reduces sensitivity and interferes with a thorough examination. Clinicians should be familiar with zoonotic diseases in their geographic area and take appropriate precautions. The hands and arms should be coated with a generous amount of a nonirritating obstetric lubricant before entry into the vaginal canal. It often is beneficial to pump 3 to 5 L of lubricant into the birth canal, in addition to applying lubrication to the operator's arms. Note that despite the long-standing doctrine of using warm soapy water during obstetric intervention, soaps are not the ideal product for the job. Although they may provide some short-term lubrication, they quickly increase the friction encountered owing to their propensity to cut fats and oils from the contact surfaces. In protracted cases with autolytic changes in the fetus, some clinicians elect to apply generous amounts of lanolin or barrier cream to the arms in order to reduce skin irritation and folliculitis, which may result from prolonged exposure to fetid tissues and fluids.

The birth canal and fetus are first examined for lesions or hemorrhage that may have been induced by previous attempts at delivery, and the caretaker is informed of their presence. The operator then determines as accurately as possible the presentation, position, and posture of the fetus and the presence, if any, of congenital abnormalities. In some cases, it is difficult to determine if the forelimbs or hindlimbs of the fetus are present in the birth canal; fetal elbow and fetal hock may have similar characteristics on palpation, which may confuse even the experienced operator. The limbs can be differentiated by starting at the hoof and counting the joints from distal to proximal up the limb. The forelimb has a carpal joint between the fetlock joint and the elbow, whereas on the hindlimb, the hock joint is palpable immediately proximal to the fetlock joint. The ears, eyes, and mandible can be used to identify the head, whereas the presence of the tail indicates a caudal presentation.

If the fetus is in dorsosacral position and the soles of the fetal hooves are directed ventrally, the limbs presented to the birth canal are the forelimbs, whereas if the soles are directed dorsally, the hindlimbs are presented first. The disposition of fetuses in transverse presentations sometimes is difficult to ascertain, and a careful examination is required. Fortunately, transverse presentation is rare in cattle, being commonly associated with fetal deformities or fetal monsters such as schistosoma reflexus.

Although cattle generally are considered to be uniparous, twins and greater multiples are not rare; thus, the number of fetuses must be determined and their appendages identified. The location of the internal septum between the two uterine horns is identified so it will not be inadvertently damaged by the wire saw should a fetotomy be selected as the method by which to relieve dystocia.

After determining the disposition of the fetus, the clinician then must determine if it is alive or dead before selecting the appropriate method to complete delivery.[24] In cranial presentation, the interdigital claw reflex can be elicited by pinching the web of tissue between the claws. A vigorous fetus responds by withdrawing the foot. A false positive result may occur if the operator mistakes movement caused by the maternal abdominal press for that of the fetus. False negative results can occur in live calves if the head and limbs are wedged in the birth canal. The swallowing reflex is elicited by applying pressure at the base of the tongue, to which a normal calf responds by swallowing or sucking. Slow or exaggerated reactions may be associated with hypoxia or may be agonal. Slight pressure on the eyeball elicits movement of the eyeball or eyelid. The eye reflex is preserved even in severely acidotic calves. The reflexes disappear in a peripheral to central progression as the condition of the fetus deteriorates. That is, the reflexes requiring the longest nerve pathways disappear before those with shorter nerve pathways as acidosis increases. Thus, the interdigital claw reflex disappears first, and the eye reflex is preserved longest. This differential loss of peripheral compared with central reflexes may aid assessment of the physiologic status of the calf.

In caudal presentation, the interdigital claw reflex of the hindlimb is similar to that in the forelimbs; however, it becomes negative earlier during the course of delivery than does the reflex in the forelimbs. Thus, the interdigital claw reflex may be negative in a viable calf, and elicitation of this reflex is a good prognostic sign. The anal reflex sometimes is used to assess the status of a fetus in caudal presentation. This reflex is elicited by pushing the examiner's finger against or into the anus. Evaluation of the response is subjective, however, and the reflex can be absent in a viable fetus.

If the fetal reflexes are ambiguous or absent, the obstetrician should examine the fetal heartbeat or umbilical cord pulse. The heartbeat can be palpated by passing the hand between the fetal forelimbs along the ventral aspect of the neck to the sternum. The fetal heartbeat is then palpable with the examiner's fingers placed on the left side of the fetal thorax. Palpation of the heartbeat in fetuses presented caudally is difficult. The normal intrauterine heart rate in calf fetuses of 70 to 120 beats per minute increases to 90 to 120 beats per minute during

delivery. The heart rate may fall to 40 to 60 beats per minute during uterine contractions. Hypoxia of the dam caused by excitement or exertion can lead to a more severe drop in fetal heart rate. Prolonged or excessive extractive force can cause the fetal heart rate to drop to near zero. As a calf becomes acidotic as a result of delayed delivery, the heart rate first increases to 140 beats per minute and then falls and becomes irregular as its condition deteriorates.

The umbilical cord of fetuses in cranial presentation is located by palpating it between the last rib and the abdomen. In caudal presentation, the umbilical cord is easily accessible. Pulsation of the umbilical vessels can be felt by applying slight pressure to the cord. The cord may be wrapped around the fetal abdomen or limb or may enter the birth canal alongside the fetus. Although not commonly required, the location of the cord can be ascertained and the cord repositioned if necessary. Pressure on the cord should be avoided during mutation of malpostures and during extraction.

Severe congestion of the head, tongue, and forelegs is the result of prolonged impaction of the fetus in the birth canal. The condition may occur in vigorous or moribund calves and does not suggest a prognosis. If delivered alive, affected calves have difficulty nursing and may require assistance plus the administration of anti-inflammatory agents.

Frequent or violent spontaneous fetal movements sometimes can be seen or felt. Exaggerated movements may be agonal, indicative of impending death due to hypoxia.

After the status of the fetus has been determined, the operator then must estimate the size of the fetus relative to the size of the maternal pelvic inlet and birth canal. Delivery by traction results in fetal respiratory acidosis, and the operator must determine if the risks of harming the dam and the fetus during extraction are justified.

For inexperienced obstetricians, effective guidelines have been formulated to assist in the decision-making process for determining if vaginal delivery is possible.[14] The basis for these guidelines sometimes is referred to as "diagnostic traction." When the fetus is in cranial presentation, dorsosacral position, and normal posture, if one person can pull the fetlocks 10 to 15 cm beyond the vulva (approximately one hand's breadth), the points of the shoulders will pass the maternal iliac shafts and the calf can be delivered vaginally if correct delivery techniques are used. When the fetus is in caudal presentation, dorsosacral position, and normal posture, if one person pulling on each leg can make the hocks appear at the vulva, the greater trochanters will pass the iliac shafts and the calf can be delivered vaginally. As experience is gained, other factors can be included in the decision-making process. For example, the chance for successful delivery by traction is increased in the following circumstances[14]:

- The head of a fetus in cranial presentation has spontaneously been brought into the pelvic inlet.
- The fetal hooves protrude from the vulva during an abdominal press and glide back into the birth canal when straining stops.
- A heifer vocalizes while applying an abdominal press—an indication that the birth canal is still being dilated.
- The fetal fetlock joints have been delivered beyond the vulva spontaneously.

By contrast, the chance for successful delivery by traction is reduced if the following conditions exist:

- The dam has not been able to spontaneously deliver the fetal head into the pelvis after a prolonged period of labor.
- The fetus is positioned in the birth canal with the forelimbs crossed—an indication that the width of the fetus at the points of the shoulders is excessive.
- The hooves are rotated with their volar surfaces directed medially—an indication that the elbows are forced together by a narrow ventral pelvic inlet.
- The fetus is so tightly lodged in the birth canal that it does not move when the abdominal press is applied—an indication that the birth canal is maximally dilated and that spontaneous delivery is not possible.

The vaginal examination is concluded by assessing the degree of dilatation of the vagina, vestibulovaginal sphincter, and vulva. Most ruptures of the vagina, vulva, and perineum occur during obstetric intervention and should be prevented by manual dilatation of the birth canal before application of traction. Manual dilatation of the caudal reproductive tract is an extremely important part of delivery technique, with many clinicians suggesting that the need for episiotomy can be dramatically reduced if time is taken to ensure that effective dilatation is achieved before application of traction. In addition, the stress and resultant acidosis in the calf are reduced, because resistance to delivery of the head and thorax is less. After application of an obstetric lubricant, the birth canal can be stretched by clasping the hands together and inserting both arms. Outward pressure with the forearms is placed in a diagonal direction with one forearm at the 2 o'clock position and the other forearm at the 8 o'clock position. Continual pressure is applied in the form of a wedge for as long as the operator can manage; then the arms are moved to the other diagonal of 4 o'clock and 11 o'clock, and the procedure is repeated. Tenacity is essential, because 10 to 20 minutes may be required to dilate the birth canal sufficiently to prevent soft tissue injury.

On completion of the examination and assessment of the condition of the fetus, the dam, and the birth canal, the clinician must then formulate a plan for resolution of the dystocia. The available options in cattle are mutation of abnormal presentation, position, or posture; forced extraction; fetotomy; pelvic symphysiotomy; and cesarean section.[16] Euthanasia may be indicated in situations in which the value of the animal is limited and the prognosis poor. When formulating a plan to deliver the fetus, the clinician must consider the value of the dam and the potential value of her offspring, the cost of the procedure and aftercare, and the prognosis for the life of

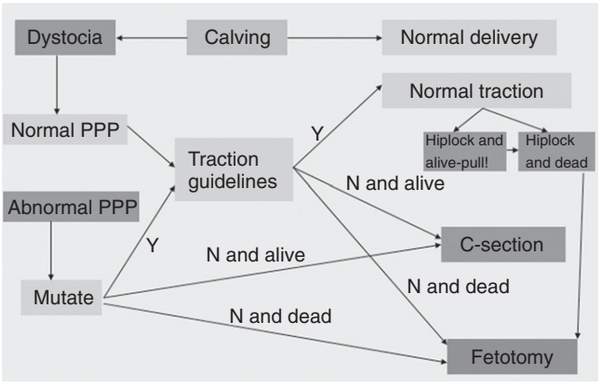

Fig. 42-3 Decision-making guidelines for dystocia case treatment (adapted from Schuijt, 1988). Y, yes; N, no. Note that a safe vaginal delivery is possible only if traction guidelines are met and correct delivery technique is used.

the dam and the fetus and for the dam's future reproductive performance. Often, the facilities and assistance available, as well as the personal preferences of the animal's owner and the clinician, will influence the decision. Figure 42-3 is a flow diagram of the decision-making process that can be used by the clinician in clinical management of dystocia.

MUTATION OF ABNORMAL PRESENTATION, POSITION, AND POSTURE

Definitions

In order to effectively communicate the details of a dystocia case, it is important to standardize terminology. In this book, the following definitions are used.

Presentation

Presentation is the relation of the spinal axis of the fetus to that of the dam. Presentation can be either longitudinal or transverse. The fetus' orientation is either **cranial** or **caudal** in the longitudinal presentation and **dorsal** or **ventral** in the transverse presentation. **Cranial longitudinal** is considered the normal presentation. Note the general trend away from the traditional use of the human descriptors of anterior and posterior in veterinary science.

Position

Position is the relation of the dorsum of the fetus to the quadrants of the maternal pelvis. These quadrants are the sacrum, right ilium, pubis, and left ilium. **Dorsosacral** is considered the normal position.

Posture

Posture is the relation of the fetal extremities (head, neck, or limbs) to its own body. Extremities may be flexed, extended, or retained (usually referring to the head). Retention can be to the right, to the left, or above or below the fetus.

Mutation

Mutation is defined as the process by which a fetus is restored to normal presentation, position, and posture by repulsion, rotation, version, or extension of extremities. Abnormalities of fetal posture generally are easier to correct when the dam is standing. In specific circumstances, however, placement of the dam in lateral recumbency can be advantageous, particularly if facilities such as a hydraulic tilt table or even a sloped loading ramp are accessible. For example, with retention of the fetal head, if the cow can be placed in lateral recumbency with the fetal head uppermost and the forequarters of the cow elevated slightly, mutation of the head to a normal posture can be facilitated. If mutation cannot be completed in 15 to 30 minutes, an alternate method for delivery should be selected.

Repulsion

Repulsion of the fetus out of the maternal pelvis into the uterine cavity where more space is available for correction is the first step in mutation. The fetus and birth canal must be well lubricated, and 3 to 5 L of a water-based lubricant can be gently introduced around the fetus through a stomach tube by means of a pump. It may be necessary to abolish abdominal straining with an epidural anesthetic, but the expulsive efforts of the dam will not subsequently be available for delivery of the fetus. In countries where it is registered for use, clenbuterol may be administered to assist in relaxation of the uterine musculature.

Care should be exercised in repelling a fetus, because uterine rupture may result from excessive pressure. Various instruments have been used for repulsion of a fetus, the most commonly used device being the Kuhn's crutch. In general, however, use of the operator's hands and arms is recommended to reduce the risk of uterine rupture below that associated with introduction of metal instruments into the uterus. In neglected cases, the uterus may be tightly contracted around the fetus, and repulsion should not be attempted.

Rotation

Rotation is defined as turning the fetus on its longitudinal axis to bring it from dorsoilial or dorsopubic position to dorsosacral position. Partial rotation also is an essential component of the routine vaginal delivery technique to ensure that the fetal hips enter the maternal pelvis on a diagonal.

In many cases, rotation can be accomplished by the hand and arm of the operator. By grasping the humerus of the ventral limb near the shoulder joint, the operator lifts the fetus upward and medially. If an assistant is available, traction on the dorsal fetal limb in a downward and medial direction can be applied to aid in rotating the fetus. Alternatively, the fetal limbs can be crossed and rotational force applied to bring the fetus to dorsosacral position. In difficult cases, use of a detorsion rod may be necessary, but excessive force that may result in injury to the dam and the fetus should be avoided.

Version

Version is defined as turning the fetus on its transverse axis into cranial or caudal presentation. Transverse pres-

entation fortunately is rare in cattle but must be converted to longitudinal presentation before delivery is attempted. Extractive force is applied to the portion of the fetus closest to the maternal pelvis while the opposite pole of the fetus is repelled. Version usually is limited to 90 degrees, and attempts to convert caudal presentation to cranial presentation are not likely to be successful and will commonly result in uterine tears.

Correction of Displacement of the Extremities

Mutation of malposture of the fetal extremities usually requires that the fetus be repelled out of the maternal pelvis before attempts at correction. In general, correction of flexion of an extremity is accomplished by repelling the proximal end, rotating the middle portion laterally, and applying traction to the distal end. Repelling and rotating forces can be applied with the operator's hand. Traction can be applied by the operator if sufficient space is available in the birth canal to permit the introduction of both arms, or by an assistant using an obstetric chain or snare.

Displacement of the Head

In cattle, the head most commonly is deviated to the left side of the fetus and lies against the thoracic wall. The malposture is corrected by grasping the orbital grooves with the thumb and middle finger (forceps grip) and drawing the head into the maternal pelvis. A rope snare placed behind the incisor teeth may be useful in difficult cases. Traction to redirect the head can be applied with the snare by the operator or by an assistant while with the other hand the operator guides the head and protects the uterine wall from the incisor teeth by covering the fetal mouth.

The head may be deviated ventrally between the forelimbs, with the mandible resting against the sternum. A hasty examination may fail to reveal the presence of the head, and the malposture may be mistaken for a case of caudal presentation. In some instances, the malposture can be corrected by repelling the fetal forehead with the thumbs while simultaneously lifting the jaw with the fingers. Correction in more severe cases requires that one or both forelimbs be repelled and flexed at the carpus, elbow, and shoulder joints. Space is then available to convert the ventral displacement of the head to lateral displacement, which is then corrected by drawing the head into the pelvis. The induced malposture of the forelimb is subsequently corrected after the head is in its proper position. Should attempts to reposition the head by these methods not be successful, the dam can be sedated, cast, and rolled to dorsal recumbency. The fetus then falls toward the maternal spine and away from the narrow ventral portion of the pelvis, allowing the head to be more easily guided into the pelvic canal.

Displacements of the Forelimbs

Unilateral or bilateral carpal flexion can be responsible for dystocia in cattle. If the flexed carpus along with the fetal head is within the maternal pelvis, the situation is described as engaged carpal flexion, whereas if the flexed carpus is cranial to the maternal pelvis it is described as disengaged carpal flexion. Correction requires that the fetus and the flexed limb be repelled cranially out of the pelvis to increase the space available for correction. The operator introduces the hand corresponding to the side of the displacement into the birth canal and grasps the metacarpus immediately proximal to the fetlock. Then the limb is lifted dorsally and the shoulder and elbow joints are flexed. When the fetlock is above the pubis, the hoof is cupped in the hand and pulled into the pelvis. If needed, traction can be applied with a snare placed proximal to the fetlock joint. While lifting and repelling the carpus with one hand, the operator applies gentle traction to draw the hoof into the pelvis with the other.

The shoulder joints also may be unilaterally or bilaterally flexed and the forelimb positioned alongside or under the fetal abdomen. Correction is accomplished by grasping the radius and pulling it toward the maternal pelvis. Shoulder flexion is thus converted to carpal flexion, which is then corrected by the methods previously described. If a traction snare can be placed distal to the carpal joint, it can be used to apply extractive force with one hand while the other repels the shoulder joint.

Shoulder-elbow flexion or elbow lock posture is most common in heifers and results in impaction of the elbow joints on the pelvic brim. The condition is recognized when the muzzle of the fetus lies directly above the hooves, rather than in its normal position approximately at the middle of the metacarpus. The malposture is corrected by first repelling the fetal body into the birth canal and then applying traction to the affected limbs, one at a time, until the elbow and shoulder joints are fully extended.

Foot-nape posture is not common in cattle but arises when one or both of the forelimbs is displaced upward to lie on top of the head and neck. The defective posture is corrected by grasping the fetlock of the affected limb and forcing it downward and laterally while simultaneously lifting and repelling the head with the other hand until the forelimbs are in their normal position. In protracted cases, continued attempts to deliver the fetus may force the hoof through the dorsal wall of the vestibule, resulting in the formation of a fistula or perineal laceration.

Displacements of the Hindlimbs

Displacement of the hindlimbs is rarely a problem unless the fetus is in caudal presentation. The incidence of caudal presentation in cattle can vary depending on management conditions and genetics, and such displacements frequently are complicated by dystocia. One or both hindlimbs may be retained and flexed at the hock or at the hip.

To correct hock flexion posture, the limb is grasped at the metatarsus and repelled cranially and laterally until sufficient space is available to draw the hoof in a caudal and medial direction into the pelvic canal. The operator should cover the hoof with one hand to protect the uterine wall as it is rotated medially. In some cases, application of a snare distal to the fetlock joint can facilitate

correction. The cord is placed between the digits of the affected hoof, and traction is applied. The operator then applies opposing forces by repelling the hock while simultaneously applying traction to the snare. This action results in flexion of the fetlock and pastern joints while the hoof is drawn toward the pelvic brim.

Bilateral hip flexion (colloquially referred to as "true breech" presentation) prevents entry of the fetus into the cervix; thus, the stimulus for the abdominal press is lacking, and signs of the second stage of labor may be minimal or absent. Hip flexion is corrected by grasping the lateral aspect of the tibia as closely as possible relative to the hock. The hock and stifle joints are flexed by drawing the hock toward the maternal pelvis. After the hock and stifle joints are fully flexed, the malposture becomes hock flexion, which subsequently is corrected as previously described.

Ventrovertical, or dog-sitting, position causes dystocia in fetuses presented cranially because of flexion of the hindlimbs at the hips. The hooves may be impacted against the maternal pelvis or lie in the vagina alongside the forelimbs. The cranial portion of the fetus is delivered normally, but the impediment is discovered when delivery cannot be completed. The condition is diagnosed by careful examination, which may be difficult if the cranial portions of the fetus occupy the pelvic canal. An attempt may be made to correct the malposture by repelling the hindlimbs as deeply as possible into the uterus. Correction is likely to be successful only when the fetus is small. Delivery by cesarean section or fetotomy may be preferable in many cases.

DELIVERY BY EXTRACTION

Early works on the topic of bovine obstetrics were written by physicians, with the first account published in 1793 and credited to Johann Günther Eberhard.[17,18] Since that time, various guidelines for delivery of bovine fetuses by extraction have been developed and modified. Advances in the science of veterinary surgery during the last half of the twentieth century have allowed veterinarians to routinely perform successful cesarean sections and have obviated the need for every case of dystocia. to be resolved by some form of vaginal delivery. The most recent obstetric guidelines are those developed and published by theriogenologists at the University of Utrecht, The Netherlands.[14]

Application of Extractive Force

During normal parturition, it has been estimated that an average force of approximately 70 kg (150 lb) is required to deliver a bovine fetus, and that 40% of this pressure is supplied by uterine contractions and 60% by the abdominal press.[19] The force required to fracture the leg of a calf varies with its weight. When traction is applied to the dorsal surface of a limb with an obstetric chain around the pastern, a mean force of 170 kg is required to fracture the leg.[20] The mean force that can be generated by veterinary students is 180 kg, with strong persons able to apply as much as 200 kg of force. A mechanical fetal extractor is capable of applying approximately 400 kg of force, and a tractor may apply greater than 5000 kg, far in excess of that considered safe. Extractive force should be applied only simultaneously with the dam's abdominal press, and tension is released when the dam ceases to strain. The abdominal press pulls the maternal pelvis into a position more nearly perpendicular to the spinal column and allows the pelvic inlet to more easily accommodate passage of the fetus. Maximum benefit from the movement of the pelvis via the sacroiliac joint is gained with the cow in lateral recumbency—one of the advantages of completing vaginal deliveries in the recumbent animal.

During a normal extraction, the force and duration of traction should not cause harm to either the dam or the fetus.[14] For this reason, the procedure should be described as "delivering" the calf, rather than "pulling" the calf. Delivering a calf implies that care has been taken to protect the health and welfare of the cow and the calf by selecting the most appropriate option for the clinical circumstances. By contrast, "pulling" a calf implies just one goal, to extract the calf, without necessarily considering the welfare of the cow and the calf. It is important from a philosophical viewpoint that veterinarians and attendants are clear on how the use of terminology can influence attitudes and behavior.

Ideally, the force applied for traction should be limited to less than the force that can be applied by one person (<160 kg). This commonly will be possible in cases in which presentation, position, and posture are normal; correct delivery techniques have been employed; and the guidelines for extraction outlined previously have been met. In a powerful extraction, the force applied is greater than for a normal extraction but is less than the pulling power of two persons (approximately 300 kg). The obstetrician needs to be aware that this amount of force has the potential to harm the dam or the fetus and that correct delivery technique such as rotation of the fetal hips before engaging in the maternal pelvis is essential. The only indication for applying the pulling power of more than two persons is in the case of hiplock of a valuable calf.

In addition to fractures of the legs,[21] improper or excessive obstetric traction has been reported to result in fractures of the ribs and vertebrae.[20,22] Calves delivered in cranial presentation also may suffer damage to the femoral nerve, with subsequent neurogenic atrophy of the muscles of the hindlimb, due to trauma associated with failure of the stifle joints to enter the pelvic canal.[23] The effect on the cow of excessive traction can include maternal obstetric paralysis, pelvic or hip fractures, and soft tissue tears of the reproductive tract. All of these potential complications negate any short-term benefit that may be gained from succumbing to the temptation to use excessive traction to effect a delivery.

Mechanical fetal extractors (Fig. 42-4) are useful devices when minimal human assistance is available. To avoid injury to the cow or the calf, however, it is essential that they be used in the manner for which they were intended. These devices are best used as a bent lever, with traction being applied to the fetus only when the cow strains. Before use, it is fundamental that the case be properly assessed and that a vaginal delivery has been

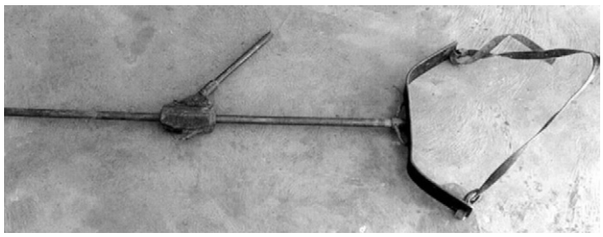

Fig. 42-4 Mechanical fetal extractors are useful for applying traction when minimal human assistance is available. To avoid applying excessive traction, such devices should be used only when diagnostic traction guidelines indicate that vaginal delivery is possible. In addition, they are designed to be used as a bent lever, with traction applied during maternal straining.

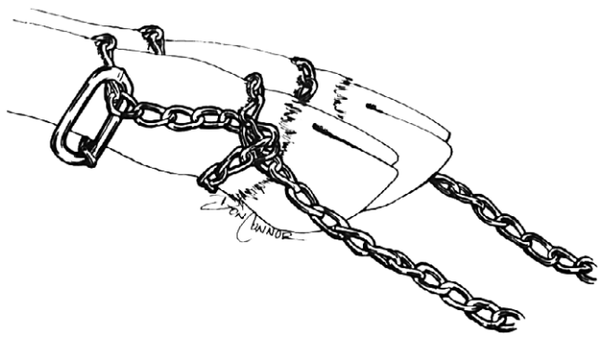

Fig. 42-5 In North America, the most common method of applying obstetric chains on a bovine fetus is to place the loop of the chain above the fetlock joint and a half-hitch around the pastern. Traction is applied to the dorsal aspect of the limb. (Original art by Mr. Don Conner, College of Veterinary Medicine, University of Missouri.)

determined to be the best option. The device is positioned on the cow and the chains from the calf's legs are attached to the hook on the ratchet. The ratchet is worked caudally along the shaft of the device until firm tension on the calf's legs is achieved. As the cow commences an abdominal strain, the shaft of the device is pushed firmly in a ventral direction to assist with the delivery. Once maternal straining ceases, the shaft is lifted dorsally and the ratchet worked to again take up the tension, in preparation for the next abdominal press.

Obstetric chains commonly are used to apply traction to the limbs of the fetus, but snares made from nylon or soft rope are equally serviceable. The main requirements with the material used to apply traction are that it can be easily cleaned, it can be easily released from the limb when traction is stopped, and it does not cause excessive trauma to the limb of the calf. The traditional method of applying obstetric chains used by many veterinarians in North America is to place a loop of the chain proximal to the fetlock joint of the fetus and place a half-hitch of the chain around the pastern (Fig. 42-5). It is thought that this practice spreads the extractive force over a larger area and may reduce the chances of injuring the fetus. Other veterinary obstetricians recommend, however, that only a single loop of chain be placed around the pastern, between the hoof and the fetlock joint.[24] In either mode of application, it is important that the large link be positioned to allow application of the force of traction along the dorsal aspect of the limb. This method of placement reduces limb fractures.

Although it is preferable for the dam to be standing during the initial examination, extraction is best attempted with the dam in right lateral recumbency. Delivering the calf with the cow in lateral recumbency is associated with a number of advantages. These include the previously mentioned movement of the pelvis at the sacroiliac joint; maximizing the potential movement of the cartilaginous pubic symphysis in heifers; and reduced gravitational effect negating the extractive force, because the operator is not required to lift the entire weight of a large fetus to bring it to the level of the pelvis. In addition, the dam can more effectively apply the abdominal press, and mechanical assistance can be applied more efficiently. An added advantage is that contamination of the area is reduced, because feces tend to fall away from the vulva and perineum when the dam is recumbent. Plastic trash bags are useful as ground sheets if the delivery is to be completed in a contaminated area.

Extraction of the Fetus in Cranial Presentation

The operator must determine if extraction is likely to be possible without endangering the dam or the fetus. The first step in delivering a fetus in cranial presentation is to position the head and extended forelimbs within the pelvic cavity. By applying traction simultaneously to both forelimbs, the strength of one person should be sufficient to pull the head into the pelvis while the dam applies an abdominal press. Traction is not placed on the head of the fetus, but the operator's hand is placed behind the head to guide it into the pelvis. The weight of an oversized calf may prevent the head from being pulled into the pelvis if the dam is standing, and it may be beneficial to cast the cow into right lateral recumbency before applying traction. If the head cannot be brought into the pelvis, a safe vaginal delivery is unlikely and an alternate method should be chosen.

After the head has been drawn into the pelvis or if delivery has progressed spontaneously to that point, the operator must determine whether continued traction is warranted by evaluating the space available between the fetal cranium and the maternal sacrum. To make this assessment, the extractive force of one person is applied simultaneously with the abdominal press, and the guidelines for extraction in cranial presentation described previously can be applied. An additional guide to the appropriateness of vaginal delivery is as follows: With the cow standing and one assistant applying traction, the operator should be able pass a hand between the cranium and the sacrum and palpate the points of both shoulder joints 10 cm or less cranial to the iliac shafts. The shoulder joints should be 5 cm or less cranial to the iliac shafts if the dam is recumbent. If the operator determines that an attempt at delivery by extraction is justified, the following procedures have been recommended.[14,25]

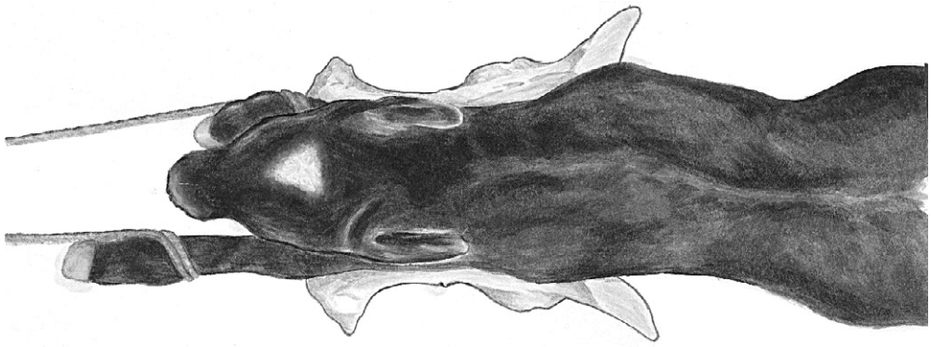

Fig. 42-6 Dorsal view of the proper application of traction for delivery of an oversized bovine fetus. Traction is applied to one limb, and that shoulder and elbow are extended. After the first limb has been extended, traction is applied to the remaining limb. Thus, the shoulders enter the maternal pelvis successively, not simultaneously. (Original art by Ms. Carmen Reed, *Dairy herd management.* Lenexa, KS: Vance Publishing, used with permission.)

The dam is cast in right lateral recumbency, and additional lubricant is pumped around the fetus if necessary. Unilateral traction is applied by one person to the left forelimb of the fetus until the shoulder is brought past the pelvic inlet (Fig. 42-6). In most instances, the shoulder can be felt to pass the ilium, but if this is not detected, it can be assumed that the shoulder has passed the ilium when the fetlock joint protrudes 10 to 15 cm (approximately one hand breadth) beyond the vulva. Then, extractive force is placed by a second person on the right forelimb until that shoulder is brought past the pelvic inlet. Delivery by traction is possible only if the second limb can be extended. If the second shoulder cannot be drawn past the ilium, the operator should elect delivery by cesarean section or fetotomy. A common error is to apply traction to both forelimbs before the shoulder joints have traversed the pelvic inlet. After both shoulder joints have passed the pelvic inlet, traction is applied simultaneously to both forelimbs in a caudal and slightly ventral direction until the head emerges from the birth canal.

The fetal hips and stifle joints constitute the next obstacle to delivery of a fetus in cranial presentation. Unfortunately, the pelvic canal of cows is oval in shape, with the greatest diameter between the sacrum and the pubis, whereas at the level of the stifle joints, the width of the fetus is greater than its height (Fig. 42-7). Thus, the fetus must be rotated from dorsosacral position to dorsoilial position to permit the widest portion of the fetal hindpart to pass through the largest diameter of the maternal pelvis. To achieve this goal, rotation of the fetus must begin as soon as the head emerges from the vulva and can be initiated by having the assistants exchange chains while they continue to apply traction. In most cases it is necessary to rotate the forepart of the fetus nearly 180 degrees to obtain the 60- to 90-degree rotation of the hindpart required for proper entry of the stifle joints into the pelvis. If sufficient rotation is not obtained by this maneuver, the operator can complete the process by wrapping the hands and arms around the fetal neck

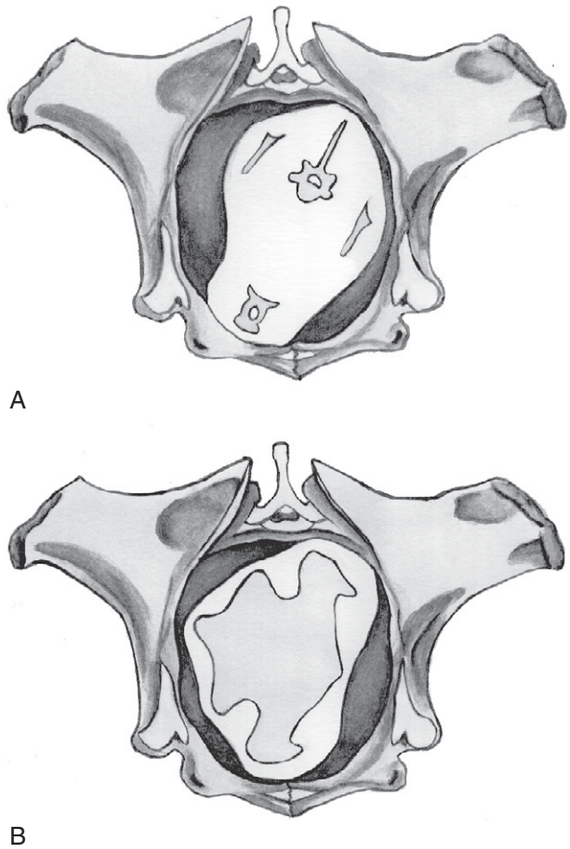

Fig. 42-7 **A,** Cross-sectional view of the fetal thorax as it passes through the maternal pelvis. The shape of the fetus conforms to the maternal pelvis, and the cranial portion of the fetus can be delivered in dorsosacral position. **B,** Cross-sectional view of the fetal hips and stifle joints as the caudal portion of the fetus is rotated into dorsoilial position to permit the largest part of the fetus to enter the maternal pelvis at its greatest diameter. Rotation of the fetus is begun as soon as its head emerges from the birth canal. (Original art by Ms. Carmen Reed, *Dairy herd management.* Lenexa, KS: Vance Publishing, used with permission.)

and applying rotational force. After the fetus has been sufficiently rotated, the operator stimulates it to breathe by clearing mucus from the nostrils and tickling the nostrils with a straw. After a normal breathing pattern has been established, traction is continued simultaneously on both limbs in a caudal and slightly dorsal direction and in concert with the dam's abdominal press.

Hiplock. Hiplock may occur despite use of proper technique, or cases may progress to that point spontaneously. In cases of hiplock, traction should be suspended and the fetus, if living, should be stimulated to breathe. An attempt should be made to palpate the fetal hindpart and determine the degree of rotation. If sufficient rotation has not been accomplished, the fetus should be repelled and rotated into position. In cases of hiplock of a living fetus, the extractive force of as many as three assistants may be applied in an attempt to deliver the fetus. Traction is applied in a caudal and slightly dorsal direction simultaneously with the dam's abdominal press. Continuous application of traction is contraindicated because the fetus has difficulty breathing while extractive force is employed and may die of hypoxia. If efforts to deliver the fetus are not successful, the fetus can be pulled sharply around toward the dam's flank. This tactic further rotates the fetal pelvis and causes one hip to enter the pelvis ahead of the other. Should these procedures fail to result in delivery, the final option is partial fetotomy or, in some instances, pubic symphysiotomy.

Extraction of the Fetus in Caudal Presentation

Delivery of a fetus in caudal presentation poses relatively more risk to the life of the fetus than does delivery in cranial presentation. With caudal presentation, the umbilical cord ruptures early, and the fetus is likely to become hypoxic and die before the head can be delivered. Therefore, delivery of fetuses in caudal presentation should be more rapid than that of fetuses in cranial presentation, and a decision should be made early on to resort to a cesarean section if the fetus is alive and delivery is impeded.

Before attempting delivery, the fetus is first rotated into dorsoilial position so that the widest portion of the fetal hindpart approaches the widest diameter of the pelvis. Rotation can be accomplished by crossing and twisting the hindlimbs. After the fetus has been rotated, two assistants apply traction simultaneously to both hindlimbs, in concert with the dam's abdominal press, in a caudal and slightly dorsal direction. Delivery is likely to be successful if the fetus can be extracted sufficiently to expose both hocks outside the vulva. If the hocks cannot be exposed, a safe delivery is unlikely and another form of delivery should be selected. After the hips have passed the pelvic inlet, the fetus is rotated back to its normal dorsosacral position by applying caudal and slightly ventral traction. When the hips have exited the vulva, the fetus is extracted as rapidly as possible, but traction is applied only while the dam strains. Excessive traction on fetuses in caudal presentation has been implicated as the cause of several injuries including fracture of vertebrae in the thoracolumbar region.

DELIVERY BY PELVIC SYMPHYSIOTOMY

In some geographic regions, the technique of pelvic symphysiotomy or pelvis splitting has been used to deliver oversized fetuses from underdeveloped beef heifers less than 26 months of age.[26] It is not indicated in older animals or in dairy heifers. Although the technique is effective, it lacks aesthetic appeal and may not be acceptable to some clients. The objective of the procedure is to split the pelvic symphysis and allow the pelvic canal to expand while traction is exerted on the fetus.

The patient is restrained with a halter and the perineal area desensitized with an epidural anesthetic. The skin ventral to the vulva is shaved and the perineal area prepared for aseptic surgery. An incision is made on the midline ventral to the vulva and deepened by blunt dissection until the caudal border of the ischium is reached. The operator places one hand in the vagina as a guide and with the other holds the blade of a heavy chisel against the symphysis. The chisel is driven cranially through the symphysis to a point 1 cm from the cranial border and the remainder of the symphysis is split by lateral pressure on the shaft of the chisel. As the fetus is extracted, the halves of the pelvis are separated and the pelvic canal is allowed to expand.

The skin incision is not closed but allowed to heal by second intention. The patient is confined to a box stall for 10 days after surgery. Antibiotics and other supportive treatments are administered as indicated.

DELIVERY BY FETOTOMY

If delivery by traction is not possible without danger to the dam or the fetus, the veterinary obstetrician must consider the option of cesarean section or fetotomy. Two basic types of fetotomy have been described in the veterinary literature. Subcutaneous fetotomy is performed with chisels and hooks, and fetal parts are removed while leaving the fetal skin to protect the genital tract and serve as points of traction. Percutaneous fetotomy is performed with a fetatome and wire saw, with sections of the fetus progressively removed.[27]

Equipment Required for Fetotomy

There are few procedures in veterinary obstetrics in which the need for experience and for proper equipment are as essential as in fetotomy. The basic equipment required is shown in Figure 42-8 and described next.

Fetatome. A two-barrel fetatome with a smooth head made from hardened steel is used. A hardened head is required to prevent the saw wire from cutting into the head during cuts parallel to or at right angles to the head. The fetatome also has a notched plate near the handle that allows fixation of an obstetrical chain under tension to maintain proper position of the instrument relative to the fetus. The ability to attach the chain to the fetatome is considered essential for efficient completion of the fetotomy procedure.

Threader and brush. A flexible shaft with an eye on one end is used to thread the saw wire through the barrels of the fetatome. Many threaders have a brush on the

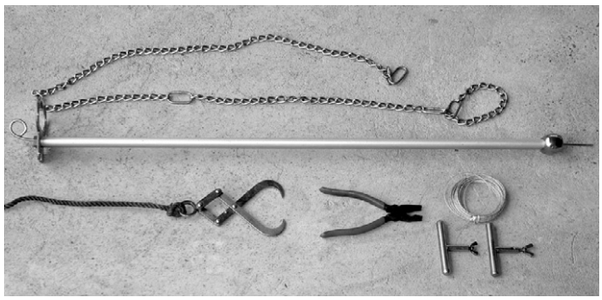

Fig. 42-8 Basic equipment required to complete a fetotomy. From *bottom left* in clockwise direction are shown the Krey hook with attached rope, Utrecht fetatome with wire threader inserted through one barrel, calving chains, a roll of fetotomy wire, fetotomy handles, and pliers. A wire introducer also can be included in the kit; however, a calving chain can be used for this purpose in most cases.

opposite end, which is necessary to clear the barrels of lubricant and debris.

Wire saw handles. Several types of wire saw handles are available, and the choice depends on the preference of the operator. Those that incorporate a spool for saw wire are convenient, but the type selected should provide a positive grip on the saw wire, be easy to attach, and be comfortable for the assistant while sawing.

Krey hook. A Krey hook with an obstetric chain or rope attached is necessary to anchor the fetatome to the fetus while making several cuts and to extract fetal parts after they have been separated.

Saw wire introducer. A curved wire introducer facilitates passage of the saw wire around fetal parts. If a wire introducer is not available, the fetotomy wire can be attached to an obstetric chain to assist in passing the wire around the fetal hindquarters.

Saw wire. A good grade of saw wire should be selected. Poorly manufactured or improperly maintained (rusted) saw wire breaks easily. Approximately 5 m of wire is required to thread the fetatome. The Utrecht fetatome is approximately 1 m long and serves as a good field guide to drawing out a suitable amount of wire. To reduce the chance of breaking the wire while sawing, it is good practice to rotate the wire through the fetatome as each cut is made and to replace the wire after several cuts.

Restraint and Anesthesia for Fetotomy

Clinicians develop personal preference for performing fetotomy with the dam either standing or in laterally recumbency. Both positions have advantages and disadvantages, and sometimes the choice is dictated by available facilities or the condition of the cow. Initially, many operators find that fetotomy is most easily performed with the patient standing in an area that allows adequate space behind the cow for manipulation of the instrument and saw wire. If the dam is recumbent and cannot be induced to rise, elevation of the hindquarters is helpful initially.

Epidural anesthesia is indicated in nearly all instances to relieve pain as well as straining. Where available, clenbuterol also should be administered to reduce the risk of uterine rupture. Tranquilization may be indicated in some instances, but general anesthesia is only rarely necessary. The administration of antibiotics before the commencement of the procedure should be considered.

Lubrication

A generous amount of lubricant is required during a fetotomy to protect the genital tract of the dam, as well as the hands and arms of the operator. Petroleum-based lubricants are preferred to water-soluble types because of their proclivity to cling to tissues and resist dilution by fetal fluids.

Assistance

At least one and preferably two assistants are required to perform a fetotomy. If one assistant is available, the clinician covers the head of the fetatome with one hand and maintains the position of the fetatome with the other hand while the assistant saws. If two assistants are available, the clinician covers the fetatome head while one assistant maintains position of the instrument and the other saws. Sawing should begin with slow, short strokes, with only light pressure on the wire. After the wire is seated beneath the fetal skin, strokes of the wire can be lengthened and heavier pressure applied. Tension on the saw wire should not be relaxed during the cutting procedure, because the saw wire may tangle and break.

Indications for Fetotomy

Percutaneous fetotomy is not a substitute for cesarean section but is indicated in certain cases of dystocia. The decision guidelines shown in Figure 42-3 are a useful aid to determining when fetotomy is indicated. Of importance, fetotomy should not be used as a last resort after the application of excessive traction when the dam and the operator are exhausted and the birth canal has been traumatized. A decision to perform a fetotomy should be made promptly after it becomes obvious that further attempts at delivery by traction are not justified. In general, fetotomy is useful to relieve dystocia caused by fetopelvic disproportion, pathologic enlargement of the fetus (fetal gigantism), incomplete cervical dilatation, fetal malposture and malpresentation, and fetal malformations including those resulting in fetal monsters. Fetotomy is not useful when the birth canal is obstructed or reduced in size, as in uterine torsion, or in instances of transverse dorsal presentation.

Percutaneous fetotomy is given primary consideration to relieve dystocia when the fetus is dead, and cesarean section is given primary consideration when the fetus is living. Exceptions may be indicated when the value of the dam and of her future reproductive ability and milk production is greater than the value of the fetus.

Either partial or complete fetotomy may be required to relieve dystocia. A complete fetotomy usually is required to deliver oversized fetuses. A complete fetotomy

following the Utrecht guidelines requires a maximum of seven cuts in cranial presentation and six or seven cuts in caudal presentation. The amputated fetal parts should be no larger than can be extracted by the obstetrician using only light traction.

Partial fetotomy is indicated in cases of fetal malposture. The offending appendage can be quickly amputated, after which the fetus can be delivered by traction. The clinician should be aware that in protracted cases, the uterus can be tightly contracted around the fetus, so that the repulsion necessary to allow mutation may result in uterine rupture. Partial fetotomy also is useful in cases of a dead fetus in hiplock that cannot be relieved without excessive traction.

Fetotomy in Cranial Presentation

Amputation of the Head

Removal of the head allows easier access to the forelimbs for amputation or mutation. A loop of saw wire is passed over the head until it rests immediately caudal to the ears. The head of the fetatome is introduced alongside the head and positioned between the mandibles and caudal to their posterior borders. If this area is not accessible, the fetatome can be positioned on the lateral surface of the face with the fetatome head caudal to the ramus of the mandible (Fig. 42-9). After the head has been amputated, it is extracted. The Krey hook can then be fixed to the exposed cervical vertebrae when needed for subsequent cuts.

Amputation of the Forelimbs

Before being amputated, the forelimbs must be extended and the distal portion of the limb protruded from the vulva. An obstetric chain is first fixed to the limb, and the chain is passed through the loop of the wire saw from above to below (Fig. 42-10).

The saw wire loop is then placed between the claws of the forefoot to temporarily anchor it. The fetatome is passed alongside the lateral surface of the limb until the head rests near the middle of the scapula. After moderate traction to extend the leg, the obstetric chain is anchored to the fetatome. The saw wire loop is removed from the interdigital space, and while the assistant applies slight tension on the wire handles, the loop of saw wire is moved up the medial surface of the limb until it lies medial to the scapula in the axillary space. The chain is then detached from the fetatome and the instrument advanced more deeply into the uterus until the head lies dorsal and caudal to the scapula. Traction is applied to the obstetric chain to fully extend the joints of the limb, and the chain is again anchored to the fetatome. The final placement is shown in Figure 42-11.

The obstetrician covers the head of the fetatome with a hand and the limb is amputated. When the fetatome has been positioned correctly, the forelimb along with the entire scapula is amputated so that the diameter of the fetus is reduced. Common errors include cutting through the shaft of the humerus or through the shoulder joint, which results in formation of sharp bone fragments and eliminates the leg as a point of traction without reducing the diameter of the fetus. The second forelimb is amputated similarly, but in most instances the procedure will be completed more easily because of the space made available by removal of the first leg.

Fig. 42-9 One method of placing the fetatome and wire in preparation for removal of the head. In some cases, this single cut will create enough room to allow mutation and completion of a vaginal delivery.

Fig. 42-10 Before placing the fetotomy wire for removal of the forelimb, the obstetric chain attached to the limb is passed through the wire loop from above.

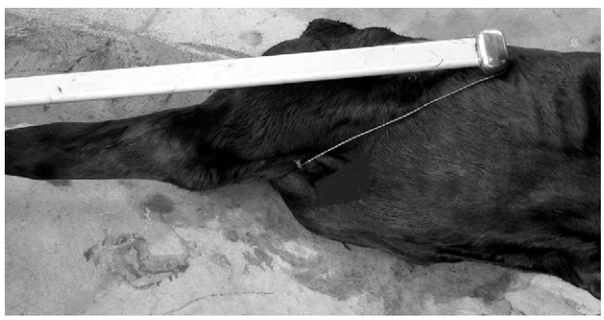

Fig. 42-11 Placement of the saw wire and head of the fetatome for an acute-angled cut to remove the forelimb.

Transverse Division of the Trunk

Two transverse cuts are made to section the fetal trunk. The first includes the neck and the cranial portion of the chest; the second cut is made caudal to the last rib. In some cases, the fetus can be partially delivered after the forelimbs have been amputated, and the first transverse cut is not necessary. In preparation for amputation of the cranial portion of the chest, the Krey hook is fixed to the exposed cervical vertebrae. The chain attached to the hook is passed from above to below through the loop of saw wire, as previously described for the forelimb. While the loop of saw wire is held externally, the fetatome is passed along the dorsolateral surface of the fetal chest until the head is near the area where the scapulas were attached. The chain from the Krey hook is then anchored to the fetatome, and while moderate tension is applied to the saw wire, the loop is positioned around the fetal thorax. The final position of the saw wire is approximately at the middle of the sternum, and the loop is at a right angle to the fetatome. Before sawing, the obstetrician should check the position of the saw wire to ensure that the fetatome was not rotated while being positioned and that the saw wires were not crossed. The fetatome tends to move up and down when sawing begins, but this motion can be minimized by pushing the fetatome against the fetus and by using short strokes with the saw wire when beginning the cut. After it has been amputated, this portion of the chest is narrow and in most instances extracted easily by traction on the Krey hook.

Amputation of the remaining portion of the chest is conducted similarly. With the Krey hook anchored to the thoracic vertebrae, the head of the fetatome is positioned on the dorsolateral surface of the fetus immediately caudal to the last rib so that the saw wire loop is at right angles to the fetatome. In the case of a large fetus, this portion of the chest may be too large to permit safe extraction. The diameter can be quickly reduced by separating the ribs from their attachment to the vertebrae. The saw wire is removed from one of the tubes and attached to an introducer. In attaching the wire to an introducer, care is required to ensure that the loose end of the knot is long enough to prevent it from acting as a cutting device, which could occur if it were left too short. After puncturing the diaphragm with a finger, the obstetrician passes the introducer and saw wire through the lumen of the thorax. The hand is withdrawn from the thoracic cavity ans reintroduced down the lateral aspect of the thorax, the introducer and wire are retrieved, and the fetatome is rethreaded. The head of the fetatome is placed near the vertebral body, and the saw wire is positioned to sever the ribs near their vertebral attachment. After the ribs have been separated, the thorax can be collapsed and easily withdrawn.

A final longitudinal division of the fetus separates the hindquarters and reduces them in size for delivery. Using an introducer, the saw wire is passed over the dorsal aspect of the pelvis and the introducer retrieved between the hindlimbs. The fetal hindpart can be divided into equal parts by placing the head of the fetatome against the lumbar vertebra or into unequal parts by placing the head of the fetatome on the lateral surface of the fetus cranial to the hip joint.

Hiplock

A partial fetotomy can be used to expeditiously relieve dystocia caused by hiplock, whether the condition arises spontaneously or is the result of inadequate rotation during delivery by traction. The forepart of the fetus is first removed by a transverse cut between the last rib and the pelvis. The hindpart of the fetus is then divided and delivered as described previously.

Fetotomy in Caudal Presentation

Amputation of the Hindlimb

An obstetric chain is attached to the limb to be amputated and passed from above to below through the loop of saw wire at the head of the fetatome. In a manner similar to that described for amputation of a forelimb, the wire loop is temporarily anchored between the claws, and the fetatome is introduced along the lateral surface of the limb and advanced until the head rests in the area of the greater trochanter of the femur. After the obstetric chain is anchored to the fetatome, the saw wire is removed from the interdigital space and advanced up the medial surface of the limb until it lies medial and cranial to the stifle joint. Traction is then placed on the obstetric chain to extend all the joints, and the head of the fetatome is further advanced until it rests dorsal to the greater trochanter of the femur. The chain is anchored to the fetatome and the limb amputated. If the fetus is very large, the second limb can be amputated similarly, or the trunk can be divided transversely with the second limb still attached if it appears that delivery of the severed portion will be possible.

Transverse Division of the Trunk

The first transverse division of the trunk is made between the pelvis and the last rib. If one hindlimb has been removed, the cut is begun by positioning the fetatome in the manner described for amputation of a hindlimb. The fetatome is positioned farther forward on the fetus, however, and the saw wire loop is positioned caudal to the last rib. This cut results in delivery of the remaining hindlimb, along with the fetal pelvis. If both hindlimbs have been removed, the pelvis is secured with a Krey hook, the saw wire loop is positioned transversely around the fetus, and the pelvis is removed.

The second transverse division of the trunk is made by positioning the head of the fetatome on the dorsolateral surface of the fetus immediately caudal to the scapulae. The saw wire loop is then positioned at a right angle to the fetatome so as to divide the fetus at approximately the middle of the sternum. If the rib cage cannot be safely extracted, it can be divided and collapsed as described for fetotomy in cranial presentation.

Division of the Forepart

The remaining fetal forepart can be reduced in size by amputation of each forelimb separately or by diagonal division of the forepart. Forelimbs are amputated by passing an introducer and saw wire dorsally between the neck and the limb and retrieving them ventrally, positioning the wire between the elbow joint and the chest. After the fetatome has been rethreaded, its head is posi-

tioned in a space that has been bluntly dissected between the scapula and the thorax. If diagonal division of the forepart is selected, the saw wire is positioned similarly, but the head of the fetatome is placed on the lateral surface of the opposite scapula. The larger portion, consisting of the cranial thorax and one limb, is delivered first, followed by delivery of the remaining forelimb and head.

Fetotomy in Abnormal Posture

Displacement of the Head

Access to the flexed neck can be facilitated by amputation of the forelimb on the side opposite that toward which the head is displaced. The saw wire attached to an introducer is then positioned between the neck and the body of the fetus. After the fetatome is rethreaded, the fetatome head is positioned as close to the thorax as possible and the neck is severed.

Carpal Flexion

If dystocia due to carpal flexion cannot be corrected by repulsion and mutation, the dystocia can be easily relieved by partial fetotomy. If the head prevents easy access to the flexed carpus, it is amputated first. Then, with the use of an introducer, the saw wire is placed around the flexed carpus and the fetatome is threaded. The head of the fetatome is positioned against the distal portion of the carpal joint. The limb is amputated and the distal portion removed. An obstetric chain can then be anchored proximal to the carpal joint for delivery by traction.

Hock Flexion

Amputation of the limb immediately distal to the hock reduces the danger of uterine rupture that may accompany attempts to repel the fetus and mutate the malposture in protracted cases. The saw wire is threaded around the limb and positioned distal to the hock joint. An obstetric chain can be anchored to the limb proximal to the hock for delivery by traction.

Hip Flexion

Using an introducer, the saw wire is passed over the dorsum of the fetus and directed between the limb and body. The introducer is retrieved ventrally and the fetatome threaded. Before the limb is amputated, the head of the fetatome is placed against the fetal ischium. In bilateral cases, it may be necessary to amputate the second limb if the malposture cannot be safely mutated.

Fetotomy for Delivery of Abnormal Fetuses

Fetal monsters are occasionally encountered as causes of dystocia in cattle. The variety of configurations will challenge the resourcefulness of the obstetrician, but fetotomy often is preferable to cesarean section.

Schistosomia Reflexus

In schistosomia reflexus, if the fetus is presented with its viscera exposed, the fetal organs can be removed after a thorough examination to ensure that rupture of the uterus has not resulted in prolapse of maternal organs. An attempt is made to encircle the fetus with the saw wire and divide the trunk near the point of deviation. Frequently the portions can then be delivered. If not, they can be reduced in size by further divisions. Sharp bone fragments frequently result, and the dam's birth canal should be protected when the fetal segments are delivered. Some abnormal fetuses are presented with three or more limbs and the head in the maternal pelvis. In these cases, an attempt is made to amputate the most accessible limb in the most expeditious manner, followed by further section of the fetus at the discretion of the clinician.

Perosomus Elumbus

In perosomus elumbus, the fetus usually is not oversized, and the fetal forepart is delivered spontaneously. Difficulty is encountered when the operator attempts to complete the delivery because the hindlimbs often are ankylosed and distorted. Attempts to withdraw the fetus by traction may result in perforation of the uterus. Delivery can be accomplished by a partial fetotomy similar to that described for resolving cases of hiplock.

Modified Fetotomy

A modification of the Utrecht method for complete fetotomy has been described that reduces the number of cuts required and is applicable in cases in which the fetus is not excessively oversized.[28]

Cranial Presentation

The head is first amputated by encircling the neck with the saw wire. The head of the fetatome is then positioned dorsal to the fetal scapula in a manner similar to that described for amputation of a forelimb, except that the saw wire is positioned between the stump of the neck and the opposite forelimb. This diagonal cut results in amputation of one forelimb, the neck, and a portion of the thorax. The resulting opening in the thorax permits evisceration of the thoracic and abdominal cavities, which further reduces the size of the fetus. In addition, the size of emphysematous fetuses is reduced by the escape of gas after breach of the body cavities. Traction on the remaining forelimb is then continued until delivery is complete or until the size of the fetal hindpart obstructs progress. If necessary, the fetal pelvis is sectioned in the manner described for resolution of hiplock.

Caudal Presentation

The first hindlimb is amputated in the manner described by the Utrecht guidelines. The fetus is then reduced in size by evisceration. If size of the fetus is not reduced sufficiently to permit delivery, a transverse cut is made through the thorax caudal to the scapula. The ribs can be severed from their attachment to the vertebrae and the thorax collapsed if necessary. A final cut is made obliquely through the remaining forepart of the fetus. One section is composed of a forelimb and most of the thorax and the other is composed of the head, neck, and remaining forelimb.

Aftercare with Fetotomy

In most cases, the dam requires less care after fetotomy than after cesarean section. On completion of a fetotomy, the uterus is routinely lavaged with warm (42° to 45°C) water to which is added a small amount of a nonirritating disinfectant or salt. Approximately 4L is pumped into the uterine cavity through a stomach tube and then siphoned out. The procedure is repeated until fetal tissue and lubricant have been removed and the efflux is clear. Dystocia is a common antecedent to secondary uterine inertia, and treatment with an ecbolic agent such as oxytocin often is indicated. Systemic antibiotics frequently are indicated, especially in cases with protracted labor. Other supportive therapy such as administration of intravenous fluids or calcium is given as required. Anecdote suggests that the uterus will contract and involute more effectively if the cow is allowed to exercise, rather than being confined in a stall. Fertility and milk production typically are higher after fetotomy than after cesarean section.

LAPAROHYSTEROTOMY (CESAREAN SECTION)

Cesarean section is an option for treating dystocia that is commonly employed when the guidelines for extraction indicate that vaginal delivery would be unsafe for the dam or fetus, and fetotomy is not a viable alternative because the fetus is alive or there is inadequate room to place the fetatome.

Cesarean section may be performed through the paralumbar fossa (most commonly on the left side), a low flank or ventrolateral incision (again, most commonly on the left), a lateral oblique incision, a paramedian incision (commonly on the right, but also can be done on the left), Marcenac's approach, or ventral midline laparotomy. The paralumbar fossa and the lateral oblique laparohysterotomy procedures usually are performed with the cow standing; all other techniques are performed with the cow recumbent. The left paralumbar fossa approach is arguably the most commonly used approach, particularly when the fetus is alive (Fig. 42-12). With this approach, no or only mild sedation is required; many owners have facilities to safely restrain a standing animal; most surgeons are more comfortable in the standing position in contrast to kneeling; and the risks associated with wound dehiscence are minimal compared with those with the more ventral approaches. Probably the greatest disadvantage with this approach is the reduced exposure of the uterus compared with the more ventral approaches. This increases the risk of abdominal contamination from leakage of uterine contents, which is a major consideration if the fetus has been dead for more than 12 hours. The paralumbar approach is not recommended for the removal of emphysematous fetuses.

The choice of sedation or anesthesia will depend on the surgical approach. Other presurgical treatments that should be routinely considered include the administration of an epidural anesthetic, clenbuterol where available, and preoperative antibiotics. In all cases, the uterus (or part of it) should be exteriorized from the abdomen

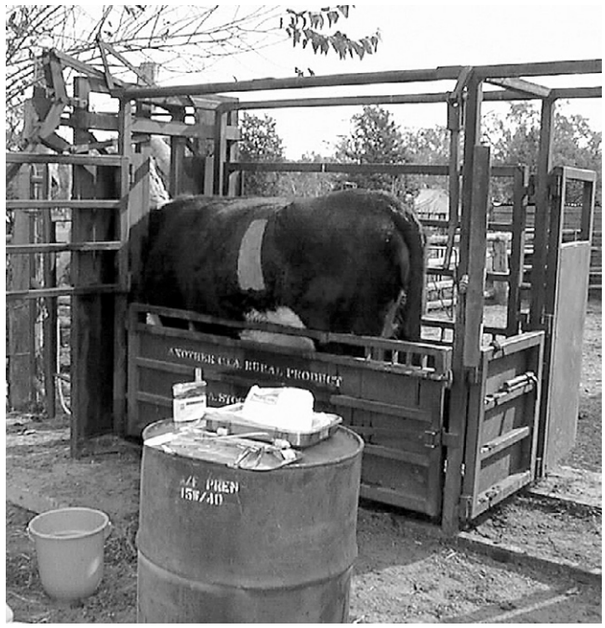

Fig. 42-12 A heifer is prepared for delivery of a calf by a left paralumbar laparohysterotomy. An inverted lumbar block and epidural anesthesia have been administered, in addition to clenbuterol and preoperative antibiotics. The left paralumbar fossa has been shaved in preparation for cleansing. No sedation was necessary in this case.

if possible. This is particularly important if extensive attempts at manual correction of dystocia have been tried or if the fetus is emphysematous. Some contamination of the abdomen is tolerated if the fetus is alive at the time of surgery. When the fetus has been dead for more than approximately 12 hours, however, the fluids within the uterus become very toxic if they leak into the abdomen.

Once the fetus is removed, the healthy uterus is closed with metric 7 chromic catgut in a double layer using the Utrecht suture pattern, which is a Cushing pattern modified so that the suture is turned to a 30- to 45-degree angle away from the incision. A double-layer closure is made so that the suture line is less likely to leak uterine fluids during postdelivery contraction of the uterus. When the health of the uterus is in question, such as with vascular damage from uterine torsion, bruising due to excessive handling, or an emphysematous calf, closure with a monofilament absorbable suture material (e.g., polydioxanone suture [PDS]) may be more suitable. These suture materials will maintain tension strength despite regional inflammation. The uterine serosa should be thoroughly lavaged clean of all blood clots before being replaced into the abdomen.

For paralumbar fossa and lateral oblique incisions, the peritoneum and transverse abdominis muscle are closed in one layer and the internal and external abdominal oblique muscles closed in the second layer using metric 7 chromic catgut suture. For ventral midline and paramedian incisions, the abdominal wall should be closed with metric 6 PDS or Vicryl suture. The far-near-near-far

suture pattern is particularly suited to closure of the bovine ventral abdomen. The skin is closed using metric 7 (special) vetafil (Braunamid) in a forward (Mayo) interlocking suture pattern.

Some surgeons lavage the abdomen (the uterus and ovary regions) with fluids containing various additives such as antibiotics, nonsteroidal anti-inflammatory drugs, and heparin before closure in an attempt to limit postoperative infection and adhesions, particularly if abdominal contamination was substantial. This precaution usually is not necessary if the surgery was routine, and there is little evidence to suggest that the inclusion of additives has any advantage over saline alone.

Oxytocin should be administered at the completion of surgery to assist with contraction of the surgical site. Antibiotics are continued for 3 days after surgery and should be directed against the most common bacteria resident in the normal postpartum uterus for the geographic region. The administration of nonsteroidal anti-inflammatory drugs for 48 hours after surgery, to control pain and limit adhesion formation, should be considered.

MANAGEMENT AND PREVENTION OF DYSTOCIA

In beef cattle production, dystocia most commonly occurs in heifers. Although results from the literature vary, it appears that common estimates of dystocia in British breed beef heifers fall in the range of 10% to 40%, with an average of approximately 20%.[29,30] In addition, dystocia is reported to be the primary cause of calf loss in 2-year-old beef heifers.[31] By contrast, among older breeders, occurrence of dystocia generally is insignificant, with estimates ranging from 0.8% to 3%.[32] Accordingly, the focus of this section of the chapter is on reducing dystocia in heifers, with some reference to older cows where relevant.

Despite the enormity and long recognition of the problem, a large deficit remains in current understanding of the causes of dystocia. For example, one prediction equation found that only 63% of the variation in calving difficulty could be accounted for when all known variables were included.[33]

Causes of Dystocia

Not a great deal has changed in the last 30 years or so regarding our understanding and classification of the general causes of dystocia in cattle. In beef cattle, these causes generally are limited owing to the relatively low metabolic pressure placed on these animals compared with dairy cattle. For example, clinical or subclinical hypocalcemia as a contributory factor to ineffective labor, or perhaps uterine torsion, is expected to be more common in dairy cattle than in beef cattle.

In decreasing order of occurrence, the three most important general causes of dystocia in beef cattle are (1) fetopelvic disproportion, (2) cranial presentation of the fetus, and (3) ineffective labor. These three causes of dystocia are reported to account for 30% to 70%, 20% to 45%, and 10% to 20% of dystocias in beef heifers, respectively.[34]

Although it is convenient and traditional to classify the causes of dystocia in this way, these broad classifications do not lend themselves to implementation of specific control measures. For example, fetopelvic disproportion simply suggests an incompatibility between the size of the fetus and the size of the maternal pelvis. The reason for the incompatibility could be one or a combination of many factors. Specific control measures can be implemented only if specific causes have been identified. In beef heifers, these specific causes usually are associated with physical traits of the heifer or calf, endocrinology of the heifer, or metabolism of the heifer.

In Australia, a recent study identified seven physical traits that influence dystocia incidence in beef heifers[35]:

- Higher mean calf birth weights
- Male calf gender
- Lower mean body weight of heifers at 12 months of age
- Lower mean pelvic area of heifers at 12 months of age
- Lower ratio of pelvic area of heifers at 12 months of age to calf birth weight
- Higher mean body condition score at calving
- Heavier placental weights at calving

In the same study, two environmental factors were identified as influencing dystocia incidence: lower mean rainfall during the second trimester of gestation for heifers bred to calve as 2-year-olds and a later mean calving date for heifers calving as 2-year-olds.

Another study focused on specific causes of dystocia associated with ineffective labor.[36] The authors described the calving difficulty experienced by heifers, which was not attributable to high calf birth weight, as "physiological dystocia." They found this type of dystocia to be associated with a deficit in precalving estradiol-17β production with consequent poor expulsive efforts. Also present may be the confounding effect of a periparturient increase in serum progesterone levels. Placental preparations from cows experiencing weak labor and requiring obstetric assistance have produced lower rates of in vitro conversion of androstenedione and pregnenolone to estrogen than those found in such preparations from cows that calved unassisted.[37] In addition, dystocia due to ineffective labor has been reported to result from deficiencies of calcium, phosphorus, copper, cobalt, selenium, iodine, sodium, zinc, magnesium, and manganese.[38]

To pinpoint endocrine imbalance as the underlying disorder, dystocia due to a large calf must be differentiated from dystocia due to ineffective labor. Estradiol-17β concentrations in plasma are positively correlated with calf birth weight.[36] It has been suggested this factor is responsible for the often increased concentrations of plasma estradiol-17β in heifers suffering dystocia due to a large calf.[39] This is in contrast with physiologic dystocia, which appears to be due to an endocrine imbalance involving low plasma estradiol concentrations, possibly in combination with high serum progesterone levels. This may stem from inadequate placental function, resulting in lower conversion rates of androstenedione and pregnenolone to estrogen in late gestation. Zinc deficiency

can reduce protein synthesis and impair estrogen-mediated responses.[40] So it also is possible that low serum zinc concentrations may be involved at this level.

Some of the aforementioned specific causes, such as high birth weight of calves, have been recognized for many years. Still others, although intuitive, have only recently been identified and may require further trials to become unequivocal.

To further complicate effective dystocia management, many underlying factors can influence how specific causes of dystocia manifest themselves. These factors can be classified in a number of ways; however, one classification method divides the factors into the following categories[7]: hereditary, nutritional, managerial, infectious, traumatic, miscellaneous, and combined. An environmental category also could be added to this list, because environmental characteristics such as temperature and humidity cannot be readily included in nutritional or managerial categories.

Identifying the Problem

Dystocia management is easier if the specific physical traits or managerial situations that influence its incidence are identified. Steps that can be taken to achieve a specific diagnosis include weighing replacement heifers at intervals from weaning to calving as 2-year-olds, measuring yearling heifer pelvic areas, weighing calves at birth, recording calf birth dates, monitoring and recording pasture quality and quantity, monitoring and recording daily rainfall figures, collecting suitable samples to assess trace element status, and recording comments for all assisted calvings. With this information, prospective and retrospective assessments can be made.

Investigations of mineral deficiency in herds experiencing dystocia associated with ineffective labor should start by analyzing 10 blood samples from heifers in late pregnancy.[38] Ideally, the blood should be collected within 2 weeks before parturition or, in the case of dystocia, at parturition. The use of postpartum blood samples may overestimate prepartum mineral levels. Whole-blood copper and glutathione peroxidase are used to assess copper and selenium status, respectively. Sample collection to determine selenium status may be an exception to the prepartum collection rule because the half-life of red blood cells affects the assessment of selenium levels. This means that blood glutathione peroxidase levels reflect not current selenium status but the animal's selenium status approximately 2 months before sampling.[40] Plasma inorganic iodine is used to assess iodine status. Plasma calcium and magnesium concentrations are used to indicate the respective levels of these minerals.[38] Serum copper and zinc concentrations also are determined.

Specific Management Practices

Several studies into the cause and management of dystocia in beef herds have been carried out.[29,30,35,41] Many general suggestions for reducing the impact of dystocia have arisen from these and other studies.[6,42,43] Ideas include changing to a breed with lower dystocia incidence, increasing surveillance of heifers during calving, delaying calving until the heifer is older than 2 years of age, manipulating nutrition at different stages of heifer development, and manipulating calf birth weight by careful bull selection. Most of these suggestions were derived from studies performed 30 years ago, and debate and confusion remain about the effects of nutrition, season, age of heifer at breeding, and pelvic area on the incidence of dystocia. Of all parameters studied and reported, high calf birth weight and male calf gender are the only ones that appear to be unequivocally linked with increased dystocia incidence.

Many specific techniques have been used in an attempt to decrease the incidence or impact of dystocia in beef herds. These are summarized next.

Objective Bull Selection

Lowering calf birth weight is one means of reducing the incidence of dystocia that remains unchallenged. With regard to selecting bulls that are to be bred to heifers, the aim is to minimize calf birth weight without dramatically reducing postpartum growth. This is easier said than done, but the use of expected progeny difference (EPD) figures can help. Even if some postpartum growth is sacrificed, the production of a live calf from a live heifer with minimal surveillance input is a beneficial outcome. In the future, the use of gene markers most likely will help to dissociate desirable traits such as low calf birth weight from undesirable traits such as low postpartum growth rates.

Limited work has been done to determine the influence of measuring pelvic area in bulls on the incidence of dystocia in heifers. Although more research is necessary, selecting bulls to produce heifers with larger pelvises appears to be a potentially useful strategy.[44] If bull pelvic areas are measured, the aim is to breed bulls with larger pelvic areas to the main cow herd so that heifer progeny have an increased pelvic area.[33]

The Use of Half-Sibling or "Littermate" Sires

In commercial herds, use of half-sibling or littermate sires appears to have given consistent reductions in the incidence of dystocia in heifers.[45] Heifers are joined to bulls that were born in the same calf crop, that is, their half-brothers. In this sort of program it is essential that the bulls be from an unassisted birth and of mean birth weight for the crop. Producers must resist the urge to select the biggest bull calf. This method of control is considered a "quick fix" only for commercial herds.

Use of Jersey Bulls

The use of Jersey bulls over heifers results in very few dystocias,[45] but also is a "quick fix" for a problem commercial herd and is not recommended as a long-term answer.

Visual Appraisal

Producers commonly use visual selection of bulls based on shoulder and pelvis conformation. It has been suggested that scapulas that slope medially from a ventrodorsal aspect are a desirable trait in bulls to be bred to heifers.[45]

Comments from field days and producer meetings indicate that many producers practice the use of visual

selection of heifers to identify those with a large frame or pelvic structure. No scientific evidence supports the effectiveness of this procedure in reducing calving difficulty. To the contrary, it has been shown that external measures of the pelvis do not necessarily reflect internal measurements of pelvic area.[33]

Use of Crossbred Heifers

A great deal of research has been carried out to compare the incidence of dystocia between purebred cattle breeds and crossbred animals.[46–51] Some of these studies refer to "specific combining abilities," which describe the likelihood of dystocia from specific crosses—for example, a Brahman dam crossed with a Hereford sire. By contrast, "general combining ability" describes the overall performance of, for example, Hereford dams crossed with any other sire type.

This research has yielded several important points:

- Calf birth weight can be significantly controlled by the dam. In this regard, dystocia incidence generally is low in Brahman cows even when bred to bulls of different breeds.[49,51] This indicates that they have a general combining ability that results in reduced dystocia.
- In Australia, purebred Poll Hereford and Hereford cattle appear to be more prone to dystocia than other breeds.[29]
- Heterosis affects heifer pelvic area, with Brahman crossbred heifers having increased pelvic areas compared with those in purebred contemporaries.[52] This may reduce dystocia incidence in crossbred heifers.
- Heterosis affects calf birth weight. Brahman-sired crossbred calves can have large birth weights as a result of the hybrid vigor associated with *Bos indicus–Bos taurus* crosses and the relative inability of *B. taurus* dams to control calf growth compared with *B. indicus* dams, as noted.

The important aspect of the last two points is that the heifer should be the crossbred animal and hence benefit from any heterosis. By contrast, if the calf is the crossbreed, heterosis may lead to higher birth weights.

Heifer Management from Weaning to Breeding

It could be argued that management of the heifer during the weaning-to-breeding period is one of the most important factors influencing subsequent calving ability as a 2-year-old. Evidence that calving difficulty in beef heifers can be influenced by energy intake and growth rates of the heifers between weaning and breeding has been available for at least 20 years.[34,53–55] One study reported a 24% increase in the proportion of heifers on a low-energy diet between weaning and breeding requirement for calving assistance compared with those on a high-energy diet.[54] Despite this knowledge, little information is available on practical methods of incorporating it into management procedures for reducing heifer dystocia. Data from a recent unpublished study suggest that British breed beef heifers should reach approximately 280 kg as yearlings in order to have greater than an 80% chance of an unassisted calving as a 2-year-old. Heifers that weigh 280 kg or more at 12 months of age will weigh approximately 320 kg three months later at joining if a growth rate of 0.5 kg/day is maintained. This mating weight is approximately 40 kg or 14% heavier than the current critical mating weight recommended for British breed beef heifers.[56] With this in mind, it is worth restating some of the relevant aspects of heifer nutrition from weaning to breeding, even though most of the research has focused on cyclicity and conception, rather than calving ability.

Heifers must reach puberty at 15 months of age if they are to conceive in time to calve as 2-year-olds. As many as 35% of all beef heifers fail to reach puberty by this time.[57] Puberty in heifers is related to height and weight; however, a minimum age requirement also must be met. The age at which heifers begin regular estrous cycles is correlated with gains in body weight from birth to puberty. It also is controlled by an array of genetic and environmental variables. The standard deviation for age and weight at puberty is considerable.[57] It is clear from the literature that variability is greatest in undernourished animals.[58] Low planes of nutrition during the prepubertal period delay puberty by inhibiting the development of a mature reproductive endocrine system.[59] An array of physiologic factors are involved; however, weight appears to be the major factor affecting age at puberty. Subsequent weight gains are needed to ensure that regular estrous cycles continue.[60] Although age at puberty is influenced by nutrition, body weight at puberty is unaffected by nutrition.[57] In addition, correlations between gains in body weight and age at puberty indicate that increased growth rate in heifers results in reduced age at puberty.[61] When winter feed levels were increased for replacement heifers,[62] it was observed that heifers on the higher plane of nutrition reached puberty earlier, had a higher rate of conception, conceived earlier in the breeding season, and experienced decreased pregnancy loss, in comparison with their lower-nutrition-plane contemporaries. These findings suggest that body weight is a useful monitor for predicting when heifers will reach puberty, so the concept of a target weight at first mating is important.[63]

Target Weights (Critical Mating Weights)

The use of target weights, or critical mating weights, for heifer selection before breeding has been recommended for approximately 30 years.[57,64] These weights give an indication of heifer maturity. They indicate when heifers are cycling and when they are mature enough to breed and will achieve acceptable conception rates in a restricted breeding period. With respect to dystocia, some evidence suggests that current recommendations may be on the "light" side.

Pelvic Area Measurements

The use of pelvimetry in both heifers and bulls is a controversial basis for programs of dystocia control.[33,65] A study in southeastern Queensland,[35] however, confirmed previous reports[33] that heifers requiring assistance at

calving as 2-year-olds had significantly smaller pelvic areas as yearlings. It also confirmed that heifers requiring assistance had smaller pelvic area–to–calf birth weight ratios. Both of these findings confirm the value of increasing the pelvic area of yearling heifers in the management of dystocia. One concern identified in the Queensland study, however, was that precise progressive measurements may be difficult to achieve using the Rice pelvimeter. This apparent imprecision suggests that the technique currently is more suited to group selection methods than to attempts to identify individual heifers that may require assistance at calving. The technique may be more useful if a more precise measuring technique is developed.

Use of a Restricted Breeding Season

The benefits of a restricted breeding season are well documented. This approach results in a defined calving period, in contrast with enterprises in which bulls are left in the herd all year. With respect to dystocia, this strategy allows more efficient surveillance of heifers at calving and provides a calf crop of similar age, to make quantitative selection of future heifers more equitable.[49] Management of nutrition for the replacement heifers is also more efficient.

Nutrition during Gestation

Studies on the effects of nutrition during gestation usually have focused on manipulating feed intake during the last trimester of pregnancy. During the last trimester of gestation, the bovine fetus gains approximately 80% of its final weight. Increased energy levels at this stage can increase calf birth weight, but the higher birth weight generally is not associated with an increase in dystocia incidence or neonatal mortality rate.[53,66–70] Restricted precalving nutrition limits calf birth weight; however, heifers on a low plane of nutrition may put less effort into the parturition process. In some trials, the feed restrictions have been severe enough to cause maternal weight loss.[68] It seems that except for extremes, attempts to reduce dystocia by manipulating nutrition during late pregnancy, in isolation from the rest of gestation, almost always fail.

A more recent study looked at the effect of rainfall throughout gestation on subsequent calving ability of extensively grazed heifers.[71] The assumption was that any effect of rainfall on heifer dystocia or calf birth weight would be through its influence on available nutrition. The findings indicated that rainfall in the first and third trimesters may have the most influence on the eventual birth weight of the calf, whereas second-trimester rainfall may be pivotal to the eventual delivery type. To summarize the findings for the whole gestation period, heifers exposed to above average rainfall in the first two trimesters followed by below-average rainfall in the third trimester had low calf birth weights and required moderate calving assistance. By contrast, heifers exposed to below-average rainfall in the second trimester followed by above-average rainfall in the third trimester had mean calf birth weights and required high levels of calving assistance. Heifers exposed to low second-trimester rainfall experienced high levels of calving difficulty.

It seems that the ideal situation is steady growth throughout gestation in the vicinity of 0.5 to 0.75 kg/day. Net growth of the heifer should be considered in the last trimester, taking into account that the conceptus is putting on 0.2 to 0.3 kg/day at this stage.

Use of Mineral or Trace Element Supplements

There is a lack of information in the literature on the effect of dietary mineral and trace element concentrations on the occurrence of dystocia in beef heifers. Dystocia due to ineffective labor has been reported to be increasing in incidence in the Irish Republic.[38] Deficiencies of calcium, phosphorus, copper, cobalt, selenium, iodine, sodium, zinc, magnesium, and manganese were identified as possibly causing increases in the duration of parturition. The correction of these multiple mineral deficiencies has been attempted using a precalving mineral supplement containing 15% magnesium, 4500 parts per million (ppm) of copper, 600 ppm of iodine, 100 ppm of cobalt, and 50 ppm of selenium, with the addition of unspecified amounts of sodium, phosphorus, zinc, manganese, and vitamins A, D_3, and E. Clinically, the use of this mix solved the problem in some cases, but a substantial number failed to be corrected, and controlled studies were not performed.[38]

In all ages of cattle, low levels of available calcium result in poor uterine muscle tone by disturbing the membrane potential across muscle cell walls. The resultant uterine inertia means that the cow has an impaired ability to expel the fetus.[38,72] Such impairment occurs when serum calcium concentrations drop below 2.2 mmol/L but is not associated with clinical hypocalcemia. Dietary control of hypocalcemia in dairy cattle is achieved by manipulating the anion/cation balance of the diet.[73] This involves reducing the precalving intake of dietary cations Na, K, and Ca and encouraging the intake of Cl and S. On an extensive production basis, the goal of reducing Na, K, and Ca intake can be achieved by prohibiting substantial grazing of extremely lush, highly fertilized grass pastures or of excessive amounts of legume; feeding only those supplements that do not contain added calcium or salt; and avoiding water that is high in Ca or Na.[40]

The role of serum zinc concentrations in the etiology of bovine dystocia has been incompletely investigated. Zinc influences hormonal synthesis, secretion, and activity.[40] Specifically, zinc can alter essential fatty acid levels and thereby affect prostaglandin synthesis. Thus, high levels of zinc may reduce $PGF_{2\alpha}$ production, which plays an important role in the initiation of parturition. Zinc deficiency can reduce protein synthesis and impair estrogen-mediated responses.[40] Initial research found a difference in the pattern of change of serum zinc concentrations in cows experiencing normal or difficult parturition.[74] A more recent trial found that mean serum zinc concentrations were significantly lower at calving in heifers requiring assistance (483 ± 28 µg/L) than in heifers not requiring assistance (655 ± 49 µg/L), despite no significant differences between the two groups during gestation.[35] Although these findings suggest that serum zinc concentrations could not be used in a predictive manner, supplementation may be considered in following years if the problem is suspected from assessing serum zinc concentrations in previous years.

The type of relationship that may exist between selenium intake and dystocia incidence is still unconfirmed. Anecdotal evidence has incriminated low selenium levels as a contributory cause of dystocia[38]; however, controlled studies have failed to confirm this assumption.[40] Recently, a small study found that heifers requiring assistance had significantly higher glutathione peroxidase activity throughout gestation than that found in contemporaries not requiring assistance.[35] The close association of iodine with selenium activity via the iodothyronine deiodinases and thyroid activity may provide a mechanism by which iodine and selenium could be associated with dystocia.

Body Condition at Calving

Some evidence in the literature suggests that dystocia resulting from "ineffective labor" may be due to overfat condition of beef heifers at the time of calving.[34,35,75] Suggestions regarding the cause of this condition have included an inability to rapidly mobilize calcium and other minerals when required[35,38]; the accumulation of fat around the vagina, decreasing its ability to dilate[34]; and an increase in fat marbling within the myometrium, possibly reducing its tone and contractility.

Recognition of this problem as a cause of dystocia correlates with the common management plan of not having heifers too fat at calving and supports the idea of including mechanisms for increasing heifer exercise in the dystocia control program.

Increasing heifer exercise. Providing exercise to heifers by forcing them to walk to water has been recommended.[76] Anecdotal evidence suggests this strategy may be useful in preventing heifers from becoming too fat during late gestation. The proposed method of action is that in addition to reducing the possibility of heifers becoming overfat, the effort also may improve uterine and abdominal muscle tone. The technique has been widely advocated throughout the industry and intuitively has merit, but currently only indirect scientific evidence is available to support its use.[75]

Time of Calving

Research on the effects of ambient temperature during late gestation on calf birth weight has produced conclusive results. Holstein calves born during the hot summer months in Florida have lower birth weights than those of calves born in the cool winter months.[77] The investigators suggested that decreased uterine blood flow during hot weather may lead to lower placental weights and less nutrient support for the fetus. Heifers calving during winter in North America are reported to experience increased dystocia compared with heifers calving during the warmer months.[7,55] More recent research involving British breed beef heifers in Nebraska supports the suggestion that cooler temperatures during the last trimester of gestation result in higher calf birth weights.[78] The authors reported mean calf birth weights 4.6 kg lighter with a 6.1°C increase in mean air temperature during the last trimester of gestation, compared with heifers calving after a cooler last trimester.

Other factors that may influence the calving time include expected rainfall and pasture conditions. This was discussed previously under "Nutrition during Gestation."

The fact that higher incidences of dystocia have been noted in heifers calving later in the calving period[35,79] may be related to natural variations in gestation length. Such variation would be expected regardless of when the calving season was scheduled. In the stud situation, accurate records of gestation length, associated with active selection, may help to reduce the occurrence of extended gestation lengths.

Parturition Induction

Parturition induction programs using corticosteroids and prostaglandins have yielded mixed results.[80] The aim is to induce calving early in order to deprive the calf of extra time to grow in utero. With the fetus growing at approximately 100 g/day during late gestation,[81] parturition would have to be induced approximately 10 days early to reduce calf birth weight by 1 kg. Unfortunately, calves born more than 2 weeks before their estimated calving date have a poor prognosis for survival in extensive management situations. The small duration between useful birth weight reduction and a nonviability of the calf means that calf losses are easily incurred using this technique. In one study in dairy cattle, 55% of induced calves either were stillborn or died before sale.[82] Problems with retained fetal membranes and the uncertainty of breeding dates in extensive situations make this an awkward method of dystocia control. Induction may potentially result in increased dystocia if used incorrectly in situations in which ineffective labor is a major problem.

Gestation Length

Gestation length is known to have an influence on dystocia incidence, but the mechanisms leading to dystocia are different for gestation periods longer or shorter than those considered normal. Gestation periods longer than 280 days may result in progressively increased calving difficulty, primarily as a result of increased calf birth weight. Gestation periods shorter than 267 days also have been found to be associated with increased calving difficulty, perhaps as a result of an hormonal environment that is not optimal for reproductive tract preparation before parturition. The prepartum hormonal environment depends heavily on the normal development of the fetoplacental unit. The length of gestation has been found to be heritable and so could be changed by selection.[66]

Increased Surveillance of Heifers at Calving

Although increased surveillance of heifers does not reduce the incidence of dystocia, this strategy can reduce the impact of the problem by controlling losses. Despite some reports suggesting that increased surveillance leads to increased dystocia,[83] the general conclusion is that potential losses probably are reduced as a result of closer supervision.[29]

THE FUTURE

Already, gene technology is allowing the identification of quantitative trait loci (QTL) that have the potential to significantly increase the rate of genetic improvement through the use of marker-assisted selection.[84] This

technology also allows antagonistic genetic correlations among traits to be broken. For example, estimates of the genetic correlations between direct effects on birth weight and yearling weight are approximately 0.5 across all breeds.[85] As a result, producers may base selection on phenotype or EPD' values, because greater yearling weight also may significantly increase birth weight, potentially increasing dystocia incidence. The converse also is true: selection for lower birth weight probably will reduce yearling weight.[84] The identification of genes or genomic regions affecting birth weight but not yearling weight will provide powerful tools to manipulate pre- and postnatal growth rates to fit individual production requirements.

The practical application of marker-assisted selection probably will mean that in the near future, a sample of blood or tail hair will allow the accurate identification of individual animals with the required traits.

The Economics of Dystocia Management

Independent estimates of the loss dystocia causes to the Australian national herd have ranged from $30 million to $48 million up to $200 million annually.[76,86,87] Even the smallest of these amounts is substantial.

Dystocia has been shown to cause significant economic loss to beef cattle properties in southeast Queensland. Although the definition of what constitutes a significant economic loss may be considered to be subjective, it could be argued that if long-term preventive measures can be instituted for less than the cost of 1 year's financial loss due to dystocia, then the economic impact of dystocia is significant.

A study based on cattle prices in 1995 found that in southern Queensland, for each percentage decrease in the dystocia incidence, the increase in gross margin was $0.13 per hectare. For the average-size beef cattle property in the study (2090 hectares), this represented an increase in the annual total gross margin of approximately $272 for each percent decrease in the heifer dystocia rate. These figures may assist decisions on how much should be spent on dystocia control methods. For example, reducing the dystocia incidence by 10% on an average-size property described above would provide approximately $2,720 extra annual income. This amount of money could help cover the cost of a set of weighing scales, or be added to the annual bull-buying budget to allow the purchase of a bull with more objective calf birth weight data.

Conclusions

Hundreds, if not thousands, of genes are estimated to be involved in the precise events leading to normal growth and development of the fetus.[88] In addition, review of the literature indicates that the causes of dystocia in beef cattle are complex and interactive. Thus, it would seem unreasonable to expect simple control methods to completely eliminate such a problem. What can be expected is to derive a set of guidelines that may be followed to alleviate the problem in the short term. This approach should be coupled with support for ongoing genetic and managerial developments to promote long-term reduction of dystocia incidence in beef heifers. The fact some breeds and individual herds are virtually free of dystocia[76] is testimony to the theory that a sustainable solution is attainable.

SUMMARY

Dystocia is a major cause of calf loss in cattle herds. Although difficult birth cannot be accurately predicted or eliminated, the effects can be reduced by improved management and skilled intervention when necessary. Replacement heifers should be well developed and fed adequately to reach 65% of their mature weight at breeding. Their nutritional needs for growth and maintenance should be met during gestation, and they should calve in good body condition. Reduced energy intake during the last trimester of pregnancy reduces calf size but does not reduce the incidence of dystocia. Dystocia may potentially be reduced by selecting service sires that have a negative EPD for birth weight; calves from sires selected for calving ease, however, suffered comparable death loss and were significantly lighter at weaning than calves sired by other bulls.[39]

Parturient cows should be observed no less frequently than every 3 hours, and delivery should be assisted if the first or second stage of labor is prolonged. Options for assisted delivery include mutation of abnormal postures, extraction, fetotomy, pelvic symphysiotomy, and cesarean section. The method selected should be effected expeditiously and proficiently and should result in damage to neither the dam nor the fetus.

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CHAPTER 43

Principles of Colostrum Feeding

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The prenatal transfer of immunoglobulins to the bovine fetus is limited by the syndesmochorial placental structure. Consequently, calves are essentially agammaglobulinemic at birth.[1] This makes the ruminant neonate totally dependent on colostrum as an immunoglobulin source. Immunoglobulin G_1 (IgG_1) is the predominant isotype in bovine colostrum, but IgA and IgM isotypes also are present.[2]

Colostrogenesis is a distinct phase of mammary gland development that clearly is regulated in part by the lactogenic hormones estrogen and progesterone. In ruminants, this phase occurs during the last month of gestation and ceases abruptly just before parturition.[3] During this period, circulating maternal IgG_1 molecules diffuse across the vascular endothelium within the mammary gland and bind to specific IgG_1 F_c receptors on the basolateral membrane of the mammary secretory epithelium. The molecules then enter the lacteal secretion by micropinocytic endocytosis.[2] Premature induction of parturition has significant implications. Prostaglandins reduce IgG concentrations, whereas corticosteroids reduce the volume of colostrum produced.[4,5] Mammalian lacteal secretions also contain nonimmunologic factors such as maternal leukocytes, lactoperoxidase, and the iron-binding apoprotein lactoferrin. Lactoferrin binds the lipid A portion of endotoxin and also sequesters iron. It is both bactericidal and bacteriostatic and may be important in neonatal immunity in the face of gram-negative infections.[6]

Neonatal survival is dependent on an interaction among the host, its environment, and management influences. Calves with failure of passive transfer (FPT) have an increased risk of death; however, many calves with FPT will survive.[7] Adequate passive transfer reduces the risk of morbidity and mortality during the first months of life. Improved productivity in feedlot animals and in dairy heifers during their first lactation also has been attributed to adequate passive immunity.[1]

FACTORS INFLUENCING PASSIVE TRANSFER

Adequacy of passive immunity in the neonate is determined by several factors. These include the age of the dam or number of previous lactations, the quality of the colostrum ingested, the volume of colostrum ingested, the timing of ingestion, and the method of colostrum administration.

Age of the Dam

It often is advocated that the colostrum from first-calf heifers has a lower IgG concentration than that of older cows and should therefore be discarded and substituted with colostrum from older cows. Several recent studies, however, failed to demonstrate any differences between colostral IgG concentration from cows in their first and those in their second or later lactations. Third-lactation cows did produce colostrum with a higher IgG concentration.[8,9] A reasonable conclusion is that calves should be fed only colostrum obtained from third-lactation cows. Third-lactation cows generally constitute only about 30% of a dairy herd, however. Consequently, in order to provide colostrum for the total calf population, each third-lactation cow would have to produce sufficient colostrum for 3.33 calves. This is an unreasonable expectation. Additionally, the magnitude of the change in colostral IgG concentrations between the first and later lactations is relatively small.[1,8]

Quality of the Colostrum

Differentiating high-immunoglobulin-concentration colostrum from low-immunoglobulin-concentration colostrum is problematic.[1] Furthermore, colostrum immunoglobulin content is highly variable across cattle breeds. Some generalizations hold true, however. Beef breed cows, such as Angus and Hereford, generally have higher colostral immunoglobulin concentrations than those in dairy cows.[2] Among the dairy breeds, Jersey and Guernsey cattle have higher colostral IgG concentrations than those in Holsteins[2,4,8] (Fig. 43-1). Pritchett and colleagues evaluated the weight of colostrum at first milking as a measure of colostrum quality. This variable correlated most highly with colostral IgG_1 concentration ($r = -0.29$). They observed that colostrum weighing less than 8.5 kg had a significantly higher IgG_1 concentration ($P = 0.0001$). Although this trend was observed in cows of all ages, it was more pronounced in the second-lactation cows.[9] In 1979, Fleenor and Stott proposed the use of hydrometry. This approach involved using the colostrometer to assess IgG_1 concentration based on the relative density of the colostrum sample.[10] Recently, however, Morin and co-workers observed that colostral specific gravity correlated more closely with colostral protein concentration ($r^2 = 0.76$) than with colostral IgG_1 concentration ($r^2 = 0.53$).[11]

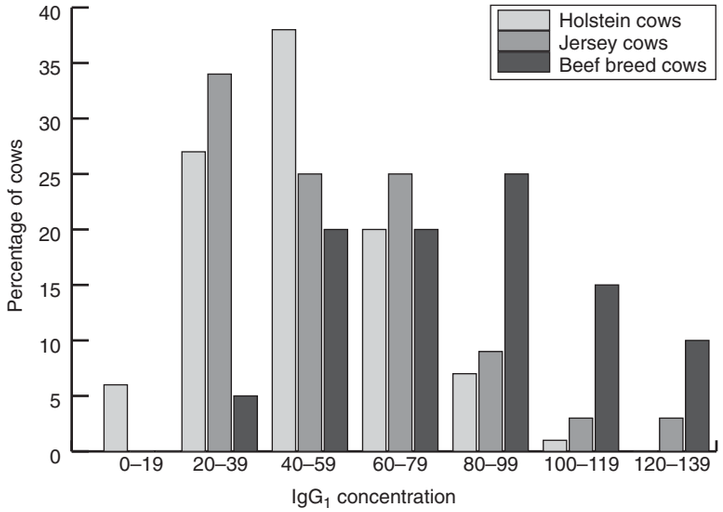

Fig. 43-1 Colostrum immunoglobulin concentrations (mg/ml) in Holstein, Jersey, and mixed-breed beef cows. (Adapted from Besser TE, Gay CC: The importance of colostrum to the health of the neonatal calf. *Vet Clin North Am Food Animl Pract* 1994;10:107–117.)

Stott and associates observed a rapid decline in all three immunoglobulin isotype concentrations (IgG_1, IgA, IgM) 12 hours after the first complete milking. In some instances, the quality of the colostrum deteriorated after the first milking, but such changes usually were seen after the second milking. Consequently, to ensure effective passive transfer, it is advocated that only the first-milking colostrum be fed to neonates.[12]

Colostral pooling, the practice of combining colostrum samples from multiple dams, is more common in dairies in which the storage of colostrum is necessary. Additionally, pooling often is encouraged in order to eliminate the deficiencies of low-quality colostrum. Of interest, colostrum produced in large volumes tends to have lower immunoglobulin concentrations, and unfortunately, it is the low-immunoglobulin, high-volume colostrum samples that will be overrepresented. In effect, they lower the pool IgG_1 concentration and hence colostrum quality. Consequently, the practice of colostral pooling is now strongly discouraged.[1,9]

Volume of Colostrum Ingested

The primary determinants for attaining adequate passive immunity are the quality and the volume of colostrum ingested. Because colostrum quality varies greatly between cattle breeds, the volume required to attain adequate passive immunity in calves also is variable. Adequate passive transfer is defined as a serum IgG_1 concentration of 1000 mg/dl in the colostrum-replete calf. To achieve this, approximately 100 g of immunoglobulin must be available in the initial colostrum meal.[2] Of interest, serum concentrations greater than 1000 mg/dl will not further decrease the mortality rate.[1]

It is currently recommended that 3 to 4 L of colostrum be delivered in one feeding to Holstein calves to ensure adequate passive immunity. The volume needed to meet IgG_1 intake goals in Guernsey and Jersey calves probably is lower.[7] The required volume greatly exceeds the voluntary intake of bottle-fed or suckling dairy calves. Besser and associates observed high rates of FPT in Holstein calves that naturally suckled their dam.[13] Consequently, force-feeding using an esophageal feeder is routinely recommended in dairy calves. By contrast, unassisted intake is an efficient method of attaining passive immunity in beef calves. Occasionally, with a less than optimal maternal-neonatal bond, human intervention becomes necessary. Adequate passive transfer generally is ensured if 2 L of colostrum is fed to neonates of the beef breeds. Force-feeding, rather than bottle-feeding, is recommended because the former is less likely to interfere with the later development of a bond between the dam and calf.

Timing of Colostrum Ingestion

Wide variations exist among calves regarding serum IgG concentrations. Time of ingestion and the amount of IgG ingested per unit body weight are the primary determinants of this variation. Colostral macromolecules are nonspecifically absorbed at the level of the ileum and jejunum of the neonatal intestinal tract. The mucosal epithelial cells that line these portions are immature and highly vacuolated and possess an apical tubular system that facilitates macromolecular absorption by a pinocytotic mechanism. The molecules then enter the systemic circulation through the venous capillaries and central lacteals of the villi. Termination of intestinal permeability or gut closure generally is age-dependent and rapidly progresses after 12 hours post partum.[5] Although immunoglobulin transfer across the gut epithelium is optimal during the first 4 hours post partum, mean gut closure time is approximately 24 hours in calves. The effect of dystocia and hypoxemia on immunoglobulin absorption has been studied. Although the absorptive capacity between normoxic and hypoxic calves was not statistically significant, gut closure was significantly delayed under hypoxic conditions (40.5 versus 20.5 hours).[14]

ASSESSING PASSIVE TRANSFER

Passive immunity is ideally assessed between 24 hours and 2 weeks of age, because endogenous immunoglobulin production and the normal decay of colostral IgG will cloud test interpretation in older calves. Several methods are available to veterinary practitioners as well as clients.[1] These vary in terms of technicality, accuracy, cost, and speed.

The gold standard for IgG_1 determination is radial immunodiffusion. Its disadvantages include the 72-hour delay for results, technical difficulty, and cost. Tests that yield results in a more timely fashion include the Quick Test Calf IgG Kit, a whole-blood immunoassay, the sodium sulfite turbidity assay, and determination of total solids by refractometry. The former test requires no prior laboratory processing, is adaptable to field use, and yields results in 20 minutes. Blood samples to be tested by the two latter methods require prior centrifugation. Such tests are easily undertaken in veterinary practices with minimal laboratory facilities, however, and yield results in a very short time. Other tests including glutaraldehyde coagulation, serum gamma-glutamyl transferase (GGT) activity, and the zinc sulfate turbidity test are not recommended because of inherent inaccuracies in test results.

CONCLUSIONS

In summary, adequate passive immunity is defined as a serum IgG_1 concentration of 1000 mg/dl. This goal is easily attainable if calves ingest adequate amounts of colostrum containing 100 g or more of IgG_1. Beef calves often will voluntarily attain adequate passive immunity. Because the quality of colostrum obtained from dairy cows is comparatively inferior, however, force-feeding is recommended in dairy calves. A volume of 4 L of colostrum is routinely administered in one feeding. In beef cattle neonates, 2 L usually is adequate.

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CHAPTER 44

Postpartum Uterine Infections

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Uterine infections are common disorders affecting dairy cows during the postpartum period. The literature is replete with case reports and retrospective and prospective studies suggesting that uterine infections decrease milk yield and subsequent reproductive performance. In these reports, however, no differentiation was made among the types of infection, and uterine infection was classified as a disease complex with clinical manifestations ranging from minimal or none to life-threatening sepsis. Consequently, a divergence of opinion continues to exist among veterinarians regarding diagnosis and treatment of uterine infections.[1]

ETIOLOGY AND DEFINITION

The uterus normally is protected from bacterial contamination by the vulva, vestibular sphincter, and cervix. During and immediately after parturition, these mechanical barriers are breached and the uterus normally is contaminated by a variety of pathogenic and nonpathogenic microorganisms. Most of these bacteria are merely transient residents and are promptly eliminated by the uterine defense mechanism during the puerperium. In some cases, however, pathogens persist in the uterus and cause disease. The organism most commonly associated with uterine disease in cattle is *Actinomyces pyogenes*.[2,3] In addition, the gram-negative anaerobes *Fusobacterium necrophorum* and *Bacteroides melaninogenicus* frequently are associated with *A. pyogenes*. *Bacteroides* decreases chemotaxis and inhibits phagocytosis by neutrophils, allowing *A. pyogenes* to persist.[4] A variety of other microorganisms occasionally are associated with uterine disease in cows and include coliforms, *Pseudomonas aeruginosa,* staphylococci, hemolytic streptococci, and others. *Clostridium* spp. occasionally infect the uterus and cause severe gangrenous metritis or tetanus. Some of the organisms that transiently contaminate the uterus during the postpartum period produce penicillinase; this should be a consideration in the selection of drugs and routes of administration used in treating the disease. In cows with a normal puerperium, the uterus is nearly free of bacterial contamination by 4 weeks after calving.[5]

Uterine infections are associated with retained fetal membranes (RFM), dystocia, delivery of twins, overconditioning, underconditioning, long-term feeding of urea to dry cows, and large herd size.[6–11] In one study, severe uterine infections frequently followed manual removal of retained membranes.[12] Unsanitary calving conditions and traumatic obstetric procedures predispose cows to uterine infections. Because of differences in their calving environment, postpartum uterine infections more commonly affect dairy cows than beef cows.

Definition of uterine infections has considered character of uterine discharge, days postpartum, clinical findings, and endocrine status.[1,13] Unfortunately, clinicians and researchers have vaguely applied terms such as metritis and endometritis when describing uterine infections, which has contributed to confusion among veterinarians in the definition and economic impact of uterine infections. Therefore, as suggested in the review by Bondurant,[13] definitions used by theriogenologists and pathologists should be applied in describing uterine infections, in order to avoid confusion. Specific definitions of related entities are presented next.

Metritis or perimetritis. Metritis is a result of a severe inflammation involving all layers of the uterus (endometrial mucosa and submucosa, muscularis, and serosa).[13] It generally develops during the first week after calving and is associated with dystocia, RFM, and calving trauma. Affected cows may be septic and present with fever, depression, anorexia, and reduced milk yield (Olsen). In addition, a copious fetid vaginal discharge may be present. Other terms used to describe metritis have included "acute puerperal metritis" and "toxic metritis."

Endometritis. Endometritis is characterized by inflammation of the endometrium extending no deeper than the stratum spongiosum.[13] This condition may follow parturition, copulation, artificial insemination, or infusion of irritants into the endometrial cavity. Usually, presence of a purulent exudate is noticed on visual inspection of the vulva. The affected animal is rarely systemically ill, and the uterus is found to be normal on palpation. Acute endometritis typically is a temporary condition, and after several estrous cycles, the offending bacteria usually are eliminated. In chronic cases of endometritis, a purulent vaginal discharge may persist. Affected cows may have a lower first-service pregnancy rate than their herdmates and require more time to conceive.[14]

Pyometra. Pyometra is characterized by a collection of purulent exudate of variable amount within the endometrial cavity, persistence of a corpus luteum, and suspension of the estrous cycle.[15] This condition is most likely to develop in cows that have their first postpartum ovulation before bacterial contamination of the uterus has been eliminated. The ensuing corpus luteum persists beyond its normal lifespan because intrauterine fluid prevents luteolysis. Progesterone continues to dominate the uterus and suppresses the uterine defense mechanism.[16] Pyometra also is an occasional clinical sign of trichomoniasis, and *Tritrichomonas fetus* should be suspected as a cause of pyometra that develops during the breeding season.

The lactation incidence of postpartum uterine infection varies among reports and with criteria used for

diagnosis. In a large retrospective study, metritis complicated 13.8% of lactations,[17] whereas the authors of a review of published literature found a mean incidence of 17.4% and a range of 8.5% to 24.2%.[18] Published reports are very difficult to interpret, however, because of the inordinate variety of criteria used to define uterine infections. Furthermore, it is likely that misinterpretation of the normal postpartum discharge from reproductive tracts of cows leads to inflated estimates of incidence of the disease.[19]

DIAGNOSIS

Clinical Signs

Clinical signs of uterine infection vary with the virulence of the causative organism and the presence of factors that predispose to the disease. Lochia normally is expelled from the reproductive tract during the first few weeks after calving, and the discharge may persist for up to 30 days if uterine involution is delayed. Normal discharges range in color from dark brown to red to white; discharge usually should not be considered abnormal unless the fluid is fetid or clinical signs of sepsis are present.

Palpation per Rectum

Examination of the uterus by palpation through the rectal wall commonly is used to evaluate the degree of involution before breeding. Palpation may not be useful to diagnose abnormalities of uterine involution during the first 2 weeks post partum, however. When involution is delayed, the uterine wall may be atonic and lack the longitudinal rugae (involution lines) typical of a normal uterus. In cases of metritis, the uterus is swollen and friable, and occasionally fibrin deposits and adhesions between the uterus and other organs or the body wall may be palpable. If involution is normal, fluid should not be palpable within the uterine lumen by 14 to 18 days after calving. In one series, cows in which a uterine lumen was palpable at the postpartum examination were likely to have pathologic changes that suggested delayed uterine involution or permanent uterine damage.[20]

A common method used for diagnosis of endometritis combines palpation of the uterus and visual inspection of the vulva for a purulent exudate. The size and consistency of the uterus and cervix, along with evaluation for the presence of fluid within the lumen of the uterus, are criteria used to diagnose endometritis. Visual inspection may reveal crust of a purulent nature formed in the perineal region. In addition, a purulent exudate may be present at the ventral commissure of the vulva.

Palpation per rectum is, however, neither a sensitive nor a specific method for accurate diagnosis of endometritis. In one trial, 157 cases of endometritis were diagnosed by palpation, but bacteria were isolated from uterine fluid in only 22% of the samples taken.[21] By contrast, evaluation of cervical size and the presence of a purulent discharge may be of diagnostic value. In a study that involved lactating dairy cows, diagnosis of endometritis was based on the presence of a purulent uterine discharge or cervical diameter greater than 7.5 cm after 20 days post partum or a mucopurulent discharge after 26 days post partum.[22] This definition was based on the negative effect that these criteria have on subsequent fertility.

Vaginoscopy

Observation of purulent exudate with the aid of a vaginal speculum has been reported to be a useful tool for diagnosis of subacute and chronic endometritis and for evaluation of response to treatment.[3,22] A prevalence of 16.9% of endometritis was found in one study, and vaginoscopy was required to identify a purulent discharge in 44% of the cases.[22] These findings suggest that visual inspection of the vulva or palpation of the uterus may not be sufficient to identify a purulent content for a confident diagnosis of endometritis. Unfortunately, the requirements for sterile and disposable specula and for aseptic preparation of the perineum and external genitals make this procedure cumbersome when cows are examined in free-stalls or lock-up stanchions.

Ultrasonography

Real-time ultrasonography has been used to demonstrate uterine changes associated with postpartum infections.[23,24] The intrauterine fluid associated with uterine infections contained echogenic particles and was easily distinguished from clear nonechogenic fluid associated with estrus and pregnancy. In addition, the uterine wall of cows affected with uterine infections was variably thickened.

Hematology

In cows with septic metritis, a degenerative left shift and marked neutropenia may occur. Hypocalcemia, which can occur early post partum, may accompany metritis. An association between ketosis and metritis has been reported in dairy cows.[25,26] Furthermore, high blood concentrations of nonesterified fatty acids (NEFAs) in cows have been demonstrated to impair lymphocyte function in vitro.[27] Thus, fat mobilization with increased blood concentrations of NEFAs may explain the higher incidence of infections observed in cows that experience an energy deficit early post partum.

Bacterial Culture

Guarded culture instruments can be passed through the cervix and samples of intrauterine fluid obtained for bacterial culture. Samples should be cultured in both aerobic and anaerobic environments. Under most circumstances, however, samples from individual animals are rarely submitted for bacterial culture because a decision to treat usually is made before results of culture are available. The cost of the procedure can only rarely be justified. *A. pyogenes* and gram-negative anaerobes usually are assumed to be the most likely causative organisms. Bacterial culture and antibiotic sensitivity tests may be indicated on farms that have an unusually high incidence of uterine infection, or if cows fail to respond to treatment.[28]

Endometrial Biopsy

Evaluation of endometrial biopsy specimens has not become as common in cows as in mares.[29] An association between isolation of *A. pyogenes* and endometrial lesions has been demonstrated.[30] Uterine biopsies at days 26 and 40, however, are reported to have a detrimental effect on future fertility.[31]

Endometrial Cytologic Studies

Neutrophils are the primary response against pathogenic bacteria of the postpartum uterus, resulting in an increase in polymorphonuclear (PMN) cells within the uterine lumen. Evaluation of the PMN count through endometrial cytology has been successful in identifying dairy cows with endometritis.[32] In clinically normal cows (without evidence of an abnormal discharge), subclinical endometritis was defined as greater than 18% PMN leukocytes during days 20 to 33 post partum and as greater than 10% during days 34 to 47 post partum. Cows with subclinical endometritis were less likely to become pregnant than cows without subclinical endometritis.

TREATMENT AND PROGNOSIS

Few topics are more controversial among clinicians than appropriate treatment for bovine uterine infections, perhaps because of the lack of precise diagnostic criteria and lack of controlled trials in which various therapeutic options have been rigorously compared. In a retrospective study, the authors concluded that the type of treatment had little effect on the outcome of the disease and suggested that only supportive therapy was needed while the patient recovered spontaneously.[33] Therapy for uterine infection has fallen into the broad categories of intrauterine therapy (antibiotics and antiseptic chemicals), systemic antibiotics and supportive therapy, and hormone therapy.[34]

Intrauterine Therapy

A variety of antibiotics and antiseptic chemicals have been infused into the uterus of cows in attempts to treat postpartum infections. Some authors have found intrauterine treatment to be beneficial, whereas others have found it to have no effect. Still others have recommended that it not be used. The bovine uterus is an anaerobic environment; thus, antibiotics chosen for intrauterine use must be active in the absence of oxygen.[35] In addition, most antibiotics and chemicals depress activity of uterine neutrophils and interfere with the uterine defense mechanism; therefore, the potential benefit of their use must be carefully weighed against their deleterious effects.[36]

Organisms that cause postpartum uterine infections usually are sensitive to penicillin, but bacterial contaminants during the first several weeks after calving produce penicillinase, which renders the drug ineffective if applied locally. By 30 days post partum these organisms usually are eliminated, and intrauterine treatment with penicillin is more likely to be effective after that time. The daily intrauterine dose recommended to reach the minimal inhibitory concentration (MIC) for *A. pyogenes* is 1×10^6 U.[37]

Oxytetracycline commonly is recommended for intrauterine therapy for postpartum infections. In a recent study, however, most isolates of *A. pyogenes* recovered from the uterus of cows were resistant to oxytetracycline, and intrauterine treatment with large doses did not affect the frequency of *A. pyogenes* isolation.[38] Furthermore, many preparations of oxytetracycline are irritating and cause chemical endometritis. If oxytetracycline is selected for intrauterine therapy, doses of 4 to 6 g/day have been recommended.

No antibiotics are approved in the United States for intrauterine administration to lactating dairy cows. Intrauterine administration of antibiotics results in contamination of milk,[39] and appropriate withdrawal times have not been determined. Assays for detection of antibiotics in milk that are available for on-farm use may be inaccurate; therefore, it is extremely difficult to ascertain whether milk from individually treated cows does or does not contain antibiotic residues.[40–43]

The use of iodine solutions for intrauterine therapy has been advocated by some veterinarians; however, there is no evidence of a therapeutic value. The incidence of RFM and endometritis was reduced in cows infused with 500 mL of 2% Lugol's iodine immediately after calving and again 6 hours later. Subsequent reproductive performance of treated cows was not reported, however.[44] Conversely, routine infusion of 50 to 100 ml of 2% polyvinylpyrrolidone-iodine solution 1 month after calving did not improve reproductive performance of normal cows and was detrimental to the fertility of cows affected by endometritis.[45] Hence, intrauterine therapy with iodine solutions for the treatment of uterine infections is not recommended.

Systemic Antibiotics and Supportive Therapy

A variety of broad-spectrum antibiotics have been recommended for parenteral administration to cows with uterine infections.[46] Penicillin or one of its synthetic analogues most commonly is recommended (20,000 to 30,000 U/kg bid). Oxytetracycline probably is not a good choice for systemic administration because of the difficulty in reaching the MIC required for *A. pyogenes* in the lumen of the uterus.

Ceftiofur is a third-generation cephalosporin that has broad-spectrum activity against gram-positive and gram-negative bacteria implicated in causation of metritis.[47] Moreover, ceftiofur has been reported to reach all layers of the uterus without violative residues in milk. Subcutaneous administration of ceftiofur at a dose of 1 mg/kg in dairy cows after parturition resulted in a concentration of ceftiofur and its active metabolites in plasma, uterine tissues, and lochial fluid that exceeded reported MIC values for common pathogens involved in metritis.[48] Smith and associates[49] demonstrated that in postpartum dairy cows affected with metritis (rectal temperature

>102.6°F, flaccid uterus and a fetid vaginal discharge), ceftiofur administered at a dosage of 2.2 mg/kg daily for 5 days, is as effective as procaine penicillin G or procaine penicillin G plus intrauterine infusion of oxytetracycline for treatment of the infection. In a multilocation study that involved 406 cows in the first 14 days post partum, ceftiofur administered at a dosage of 2.2 mg/kg daily for 5 days was efficacious in the treatment of metritis (rectal temperature >103.1°F with a fetid vaginal discharge).[47] Ceftiofur is approved in the United States for systemic administration to lactating dairy cows affected with metritis.

If dehydration complicates metritis, appropriate fluid therapy should be instituted and may be life-saving. Depending on the degree of dehydration, oral or intravenous administration of a polyionic nonalkalizing solution is indicated. Nonsteroidal anti-inflammatory drugs such as flunixin meglumine are used to combat toxemia and improve appetite. Furthermore, cows with metritis may experience depressed appetite, affecting calcium and energy status. Consequently, therapy with calcium and energy supplements may be warranted.

Hormone Therapy

A variety of hormones have been administered to cows in attempts to prevent or treat postpartum uterine infections. Estrogen has been administered to initiate or strengthen myometrial contractions, but its use is controversial. Contractions induced by estrogen have been blamed for forcing the septic contents of the uterus not only through the cervix but also into the uterine tubes, to result in severe bilateral salpingitis.

Oxytocin causes contraction of the myometrium if the organ is dominated by estrogen. Thus, oxytocin is expected to be effective in aiding uterine evacuation if administered within 48 to 72 hours after calving. Doses of 20 to 40 U repeated every 3 to 6 hours are commonly used.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its synthetic analogues have been widely used to treat a variety of abnormalities of the reproductive tract, including postpartum uterine infections.[50] During the immediate postpartum period, serum concentrations of $PGF_{2\alpha}$ and its metabolites are elevated; these increases are thought to be related to the process of uterine involution.[51] Administration of exogenous prostaglandin during the postpartum period does not alter the rate of uterine involution, however, nor is involution retarded when prostaglandin synthesis is inhibited. $PGF_{2\alpha}$ is a potent luteolysin perceived by some investigators to have an additional beneficial effect on the pituitary, affecting resumption of postpartum cyclicity. Prostaglandin treatment did not have any effect on the time required for cows to resume ovarian activity after calving, however, and had no consistent effect on plasma luteinizing hormone concentrations.[52] Several clinical trials have shown that administration of prostaglandin during the postpartum period may enhance the reproductive performance of dairy cows that are otherwise unaffected by periparturient diseases.[53,54] Likewise, cows affected with dystocia, RFM, or both and treated with $PGF_{2\alpha}$ early post partum, followed by a second treatment of $PGF_{2\alpha}$ 14 days later, experienced a higher conception rate to first service than did untreated cows experiencing a normal or abnormal parturition.[55]

Prostaglandin is the agent of choice for therapy of pyometra. Treatment is followed in 3 to 9 days by evacuation of the uterus in 85% to 90% of treated cows. After endometrial lesions are allowed 30 days to heal, fertility is restored in most patients.[56]

Pituitary responsiveness to gonadotropin-releasing hormone (GnRH) is low at parturition but increases until sufficient luteinizing hormone to induce ovulation is released by administration of GnRH at approximately 2 weeks post partum in milked cows.[57] GnRH treatment at 2 weeks post partum has been shown in some clinical trials to improve reproductive performance of cows affected by puerperal abnormalities such as dystocia, retained placenta, uterine infections, hypocalcemia, and ketosis.[58] The mechanism by which reproductive performance might be improved by treatment with GnRH is unclear, because treatment with GnRH at 15 days after calving neither hastened uterine involution nor reduced bacterial infections of the uterus.[59] Induction of ovulation during the early postpartum period with GnRH may have some negative effects on reproductive performance. Longer intervals to first estrus and pregnancy followed treatment with GnRH in a herd affected by an above-average number of postpartum uterine infections because of increased pyometra and prebreeding anestrus.[60] Furthermore, treatment with GnRH early post partum, alone or in combination with $PGF_{2\alpha}$ 14 days later, which potentially may have induced cyclicity, did not improve reproductive performance in dairy cows affected with dystocia, RFM, or both.[55]

The prognosis for recovery from postpartum uterine infections varies with severity of the condition. Most cows with uncomplicated endometritis can be expected to recover. Metritis complicated by septicemia may result in permanent impairment of fertility, decreased milk yield, laminitis, or, in extreme cases, demise of the patient despite aggressive treatment. Pyometra is rarely accompanied by abnormal clinical signs other than anestrus in cows and rarely endangers the health or life of the animal. Most cows recover promptly from pyometra if the condition is diagnosed and treated early in its course.

The cost of a case of metritis has been estimated to be $106,[61] and most clinicians assume that metritis has a negative influence on subsequent reproductive performance of affected cows.[18] In only one of four herds studied, however, did cows with metritis treated systemically with oxytetracycline have reproductive performance inferior to that of their unaffected herdmates.[62] In another large retrospective study, metritis was found to affect neither milk yield nor subsequent fertility of cows in their first lactation.[63] Likewise, a study that evaluated data from 37,776 Finnish Ayrshire dairy cows determined that metritis had no effect on milk yield when considered as a one-disease complex.[64] When early and late metritis events were analyzed separately, however, the time of disease occurrence affected milk

yield. Late metritis was not associated with milk loss. By contrast, early metritis (within 28 days post partum) significantly reduced milk yield, as indicated by monthly-test-day milk yields.[64]

PREVENTION AND CONTROL

Cows affected by periparturient abnormalities such as hypocalcemia, dystocia, and RFM are more likely to contract uterine infections than are cows that calve normally. Thus, management of sanitation, nutrition, population density, and stress to prevent or reduce the incidence of these predisposing factors should be impeccable. The most sanitary environment possible should be provided for calving, and strict attention paid to asepsis when relief of dystocia is necessary. Routine treatment of cows with antibiotics after calving has been shown to reduce fertility.[65] Excessive contamination of the environment with pathogenic microorganisms has resulted in infection of the reproductive tracts of cows during the second and third months post partum. Usual sanitary procedures did not appear sufficient to prevent spread of infections, and isolation of cows with purulent discharges was recommended.[66]

Management of RFM commonly involves an intrauterine antibiotic regimen to prevent the development of metritis.[19] Alternatively, the prophylactic use of systemic antibiotics in cows affected with RFM to prevent metritis may be considered. Systemic administration of ceftiofur in dairy cows affected with dystocia, RFM, or both reduced the incidence of metritis by 70% compared with cows not treated with antibiotics or those treated with estradiol cypionate (ECP).[67]

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CHAPTER 45

Retained Placenta

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DEFINITION

Primary retention of the fetal membranes results from a lack of detachment from the maternal caruncles, whereas secondary retention is related to a mechanical difficulty in expelling already detached fetal membranes (e.g., uterine atony). Primary and secondary retention mechanisms can coexist.

Physiologic versus Pathologic Retention

Theoretically, all cows that calve have retained fetal membranes (RFM). Greater than three fourths of cows, however, expel their placenta by 6 hours, and very few cows after 12 hours post partum (Table 45-1). Detrimental effects on reproductive performance, milk production, postpartum disease, and culling rate were detected when duration of retention exceeded 12 hours (Table 45-2). For fourth parity and higher, the cows' overall performance was best with expulsion of the placenta within 6 hours post partum. For this reason, it has been proposed that, in older cows, retained placenta is a failure to expel placenta within 6 hours after parturition. Heifers that expelled their placentas within 6 hours after calving had the lowest incidence of mastitis occurring within 1 week after calving. Because the incidence rates of RFM and postpartum disease vary with parity, the definition of retained placenta also may be age-dependent or parity-dependent. Periods from 8 to 48 hours have been proposed by various authors, but 12 hours is a widely used lapse to define RFM.

Duration of Pathologic Retention

In one series, spontaneous expulsion of retained membranes occurred between 5 and 7 days post partum in greater than one half (59%) of cows with RFM (Table 45-3). In a different experiment, however, when detachment was tested by manual traction on the membranes, they were found to be loose and "floating" in the uterus between 2 and 4 days post partum in 50% of cows with RFM. It seems that in some cows, a delay of 1 or 2 days may occur between detachment and expulsion of retained membranes. Delayed expulsion was more frequently observed in cows induced to deliver with dexamethasone (70%) than in cows with naturally occurring RFM (40%). Only 6% of the cows retained fetal membranes for 2 weeks or more. A mean retention duration of 6.8 days (range, 2–11 days) was reported. Distribution of the values for duration of retained placenta was reported to be bimodal, with a first peak at day 3 post partum and a second peak by day 7. One explanation for the bimodal distribution in expulsion of RFM is that cotyledon proteolysis was the detachment mechanism by day 3, and caruncle necrosis by day 7 post partum.

EPIDEMIOLOGY

Incidence, morbidity, and mortality rates are shown in Table 45-2. An average incidence frequently reported for a relatively large number of calvings has been shown to range from 4% to 11%. A comprehensive study of about 369,000 calvings showed the incidence of RFM to be 6.6% after all births and 4.1% after normal deliveries only. Risk factors that are known to affect incidence rates are listed in Table 45-4. Notice the high risk factor values in conditions in which both physical damage to the uterus was inflicted and hormone imbalances were induced. Also of interest is that eradication of brucellosis did not statistically decrease RFM incidence.

ECONOMICS

In a study conducted in The Netherlands with 160,000 calvings, the relative economic impact expressed in percentage was identified in four main areas: decreased milk production (40%), increased veterinary services (32%), increased culling rate (19%), and increased calving interval (9%). In other studies in Holsteins in the Northeast United States and in Ayrshires from Finland, however, retained placenta had a significant effect on milk yield. For example, it was calculated that second-parity cows decreased milk production by 1.4 kg/day as a result of retained placenta. In these same studies, it also was estimated that retained placenta lowered conception rate by 14%, compared with that in cows free of this disorder.[1]

Information concerning the cost of RFM in the United States is not readily available. For cows treated by a veterinarian, a cost of $244 per affected cow, or about $154 million per year, was estimated. Perception of economic losses per affected cow varied between veterinarians ($100 to $200) and producers ($150 to $300) in East Tennessee. It is possible that our calculations (1995) represent an overestimate, because the cost for a lactation with metritis was estimated at $106 in a 1986 study conducted in Michigan, and total health-related costs for 1990 in Ohio were $188 per cow. Herds with RFM incidence of 30% but not with 10% are considered problem herds.

Table **45-1**

Timing of Placental Expulsion after Parturition*

Hours Postpartum	% of Cumulative Expulsions
3	16.0
6	77.3
9	88.7
12	94.6
15	96.2
18	97.8
21	98.5
24	100.0

*During 3-hour time intervals. *N* = 871. Only cows expelling placentas by 24 hours or less were included. Values are approximate.
Data from van Werven T, et al: *Theriogenology* 1992;37:1991.

Table **45-2**

Biomedical Effects of Pathologic Retention of Fetal Membranes in Cows

Factor	Change*
Physiologic	
Appetite	Decreased in 60% of cows
Uterine involution	Delayed 11 days
Uterine chemotaxis	Decreased
Uterine immunity	Decreased
Milk volume secreted	(0) % to (–) 2%
Milk chemistry	Fat, protein unchanged
Uterine bacteria	Increased
Reproductive Management	
Return to heat	Delayed 17 d, 19 d
Number of inseminations	(+) 15%
Conception rate	(–) 11%, (–) 19%
Calving interval	(+) 10 d, (+) 19 d, (+) 20 d
Culling rate	(+) 5.2%, (+) 7.9%, (+) 10.5%
Milk production	(–) 207 kg, (–) 200 kg, (–) 168 kg
Days open	(+) 26 d, (+) 31 d
Overall performance	Best when retention is less than 6–12 h
Health-related	
Metritis	(+) 18%, (+) 23%, (+) 28%, (+) 53%
Lochiometra	(+) 20%
Mastitis	(0) %, (+) 5.8%, (+) 10%, (+) 15%
Previous retention	(+) correlation
Cystic ovary	(+) 0%, (+) 15%, (+) 50%
Ketosis	(0), (+) correlation
Epidemiologic	
Incidence	Average 7.5% (2–55%)
Effect of season	(+) 2%, (+) 5%, (+) 12% summer
Effect of year (1976–1983)	1.6% curvilinear increase in 7-year period
Morbidity	1.96–55%
Mortality	1–4%
Effect of age/parity	Positive correlation

(0) (+) (–), unchanged, increased, or decreased compared with cows without retained fetal membranes.
*Range or values reported by different researchers. Because of inconsistent experimental designs, data cannot be extrapolated between studies.

ETIOLOGY

Detachment of the fetal membranes indicates that uterine involution is progressing normally. Involution of the uterus is accompanied by a massive breakdown of collagen and other proteins. Lack of cotyledon proteolysis (collagenolysis) appears to be the underlying cause of RFM. If placenta-anchoring systems are not enzymatically degraded, fetal membranes are retained. Analysis of risk factors (see Table 45-4) may help to identify causes.

Anchoring Systems

The fetal cotyledon engulfs the maternal caruncle to form the primary anchoring system of the placenta, which keeps maternal and fetal tissues in apposition. Cotyledons form a cul-de-sac (pouch) that fits perfectly around the caruncle, and the open end simulates a purse-string closure (Fig. 45-1). Secondary anchoring mechanisms involve the rootlike penetration of caruncle crypts by cotyledon villi and the adhesive fluid present in the fetal-maternal interface (Fig. 45-2). The main function of the secondary anchoring mechanism is to hold together maternal and fetal epithelia within the placentome for physiologic exchange. By itself, the secondary anchoring mechanism seems to be capable of maintaining the fetal-maternal attachment throughout pregnancy, as observed in placentomes that are flat, without engulfment of the caruncle by the cotyledon (see Fig. 45-1).

The Cotyledon-Caruncle Detachment Process

Morphologic Events

Detachment of placenta in the cow involves separation of the finger-like cotyledon villi from the caruncle crypts without significant tearing of either fetal or maternal epithelia. By contrast, in the ewe, a breakdown of the cotyledon epithelia ensues when the placenta separates and, in invasive types of placentation, detachment occurs at the uterine matrix. These findings suggest that collagenolysis and proteolysis during placental detachment break apart link number 1 in the ewe and link number 2 in the cow (see Fig. 45-2).

For the cotyledon villi to separate from the caruncle crypt, it is critical that the mouth of the cotyledon "pouch" be opened first by proteolytic enzymes. This is achieved either by following three or four natural lines starting from the "mouth" of the cotyledon toward the apex (dehiscence) or by following a concentric band pattern in which the edge of the cotyledon "pouch" is digested first (Fig. 45-3).

After placental detachment is accomplished, uterine involution is completed in an average of 39 days in normal cows and 50 days in cows with RFM. By day 6

Table **45-3**

Duration of Retention*

Retention (d)	% Expelled (Cumulative %)	% Retraction (Cumulative %)
2–4	32 (32)	50 (50)
5–7	27 (59)	38 (88)
8–10	28 (87)	8 (96)
11–13	7 (94)	4 (100)
14–17	6 (100)	—

*Estimated by either spontaneous expulsion of membranes or manual retraction of hanging membranes.
Adapted from van Werven T, et al: *Theriogenology* 1992;37:1191; and Eiler H, Hopkins FM: Successful treatment of retained placenta with umbilical cord injections of collagenase in cows. *J Am Vet Med Assoc* 1993;203:436.

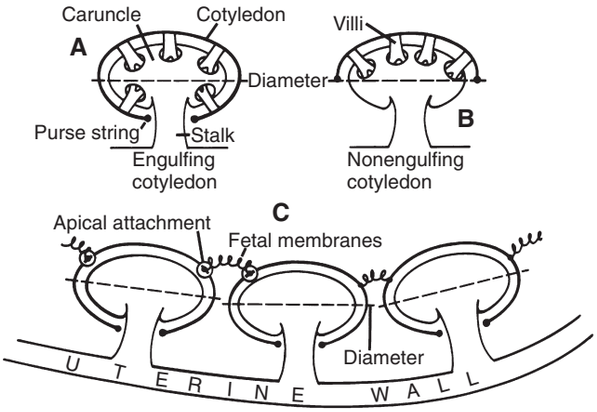

Fig. 45-1 Anatomy of the anchoring mechanism hypothesis. **A,** Cotyledon exceeds the diameter of the mushroom-like caruncle dome and engulfs the caruncle. This feature has the effect of a purse string, securing the cotyledon around the caruncle stalk. **B,** Conversely, the cotyledon ends at the maximal diameter of the caruncle dome, without engulfing the caruncle. **C,** Fetal membranes have apical attachments to the cotyledon, distal to the maximal diameter of the caruncle dome, preventing exertion of excessive pulling force on the easy-to-tear edge of cotyledons. When membranes are under tension, the oval shape of the placentome favors the formation of divergent folds. This anatomic arrangement decreases the risk of cotyledon detachment during pregnancy and makes manual removal of retained fetal membranes more difficult. (From Eiler H, Hopkins FM: *J Med Vet Assoc* 1993;203:436–443.)

Table **45-4**

Association of Obstetric, Physiologic, Hormonal, Nutritional, and Infectious Factors with Retained Placenta in the Bovine

Factor	% Retained Placenta	Relative Risk*
Obstetric		
Abortion	62	10.3
Multiple birth	37	8.3
Two previous retentions	25	6.0
Cesarean delivery in hospital	62	6.0
Stillbirth	19	4.4
Fetotomy	26	4.1
Advanced age of cow	10	3.3
Cesarean delivery	26	3.2
One previous retention	12	3.0
Difficult calving	13	2.1
Physiologic		
Short gestation plus low calf weight	12	3.0
Summer calvings	11	1.6
Sex of calf (male)		1.05
Hormone Imbalance		
Prepartum ovariectomy	100	15.1
Prepartum corpus luteum ablation	100	15.1
Abnormal (high/low) prepartum		
Progesterone	90	13.6
Estrogens	34	5.1
Induced delivery		
Prostaglandin $F_{2\alpha}$	80	12.1
Dexamethasone + prostaglandin $F_{2\alpha}$	79	12.0
Dexamethasone	67	10.1
Dexamethasone + estrogens	67	10.1
Dexamethasone + relaxin	15	2.2
Nutritional		
Selenium/vitamin E deficiency	23	2.4
Feeding hay crop/corn silage	28	1.8
Excess iron	16	1.5
Infectious		
Brucellosis-positive status of cows	28	3.0

*Calculated by dividing the proportion of cows with the factor of interest (column 1) that caused retained placenta by the proportion of cows without the factor. An error in the estimates is possible owing to variation in experimental designs.

post partum, caruncle septa are disorganized; by day 15, caruncles are completely sloughed as a result of necrosis. Consequently, RFM are detached by caruncle necrosis within 6 to 10 days and not later than 17 days post partum (see Table 45-3). The surface of the endometrium is covered by new epithelium by day 26 to 30 post partum.

Biochemical Events

Postpartum uterine biochemistry is dominated by increased collagenase and other protease activities that correlate with different stages of parturition, resulting in a massive breakdown of collagen and other proteins during uterine involution. As a result, the weight of the cow's uterus decreases from 9.0 kg at parturition to 1.0 kg at 30 days post partum.

Physiologic Events

Physiologic release of placenta is accomplished in most cows between 3 and 6 hours post partum. Cotyledon proteolysis (dehiscence) and decreasing adhesiveness (viscosity) of the cotyledon-caruncle interface fluids seem to

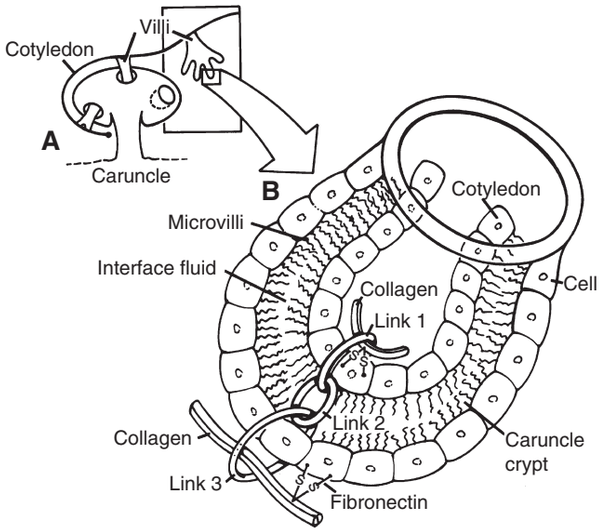

Fig. 45-2 Cotyledon-caruncle interlocking mechanism hypothesis. **A,** Root-like cotyledon villi are housed in caruncle crypts. Villi can be easily detached from the caruncle crypt by pulling from the edge of the cotyledon. **B,** Components of the fetal-maternal interface are the fetal (cotyledon) and maternal (caruncle) epithelia, epithelial attachment fibrils (perhaps fibronectin) to their own collagenous matrices, and the adhesive interface fluids between microvilli of both epithelia. The fetal-maternal interface is held together as if by three links of a chain. Link 1 is the binding of the cotyledonary epithelium to its matrix collagen. Link 2 is the gluing action of the proteinaceous interface fluids that join the fetal and maternal epithelia. Link 3 is the binding of caruncle epithelium to its matrix collagen. Hypothetically, disruption of any of the three links would cause release of fetal membranes. Conversely, persistence of the three links would cause retention of fetal membranes. (From Eiler H, Hopkins FM: *J Med Vet Assoc* 1993;203:436–443.)

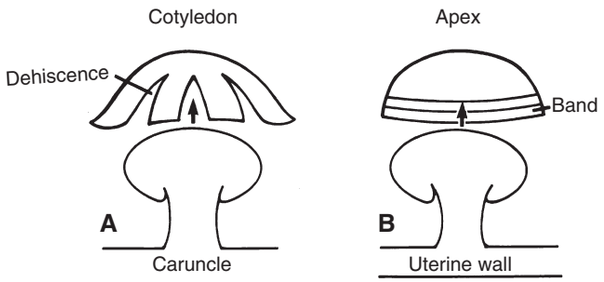

Fig. 45-3 Cotyledon proteolysis hypothesis. **A,** Proteolytic enzymes, most likely collagenase, may cause dehiscence of engulfing cotyledons by hydrolysis of cotyledon tissues towards the apex from the periphery, following three or four natural lines. **B,** In nonengulfing cotyledons, proteolysis progresses in concentric bands from the periphery to the center of the placentome, with or without dehiscence. Proteolysis opens the cotyledon, causing release of the caruncle. Lack of cotyledon proteolysis would result in retention of fetal membranes. Ultimately, retained membranes would be released by caruncle necrosis. (From Eiler H, Hopkins FM: *J Med Vet Assoc* 1993;203:436–443.)

be key factors in the release of placenta. Collagenases are capable of reducing the specific viscosity of collagen. Collagenase activity of cotyledon villi during delivery is increased in healthy cows and decreased in cows with RFM. The cellular sources of collagenase and proteolytic enzymes for placental release in the cow are unknown. In laboratory animals and humans, myometrial cells, fibroblasts, and leukocytes have been identified as sources of collagenase in the uterus. Lack of uterine motility is not considered as a reason for primary retention, because uterine motility is normal or above normal in cows with primary RFM.

Trigger of Uterine Involution Proteolysis

Serotonin, abundant in fetal blood but not in neonatal calf blood, causes release of collagenase by uterine cells. Serotonin has been proposed as a signal to begin the massive collagen degradation that occurs in the postpartum uterus. It has been suggested that the roles of serotonin during delivery are to stop blood circulation between the placenta and the fetus and to trigger uterine proteolysis.

Furthermore, uterine distention by the fetus has been proposed as an inhibitory factor to prevent uterine involution. In rats, regression of a pregnant uterine horn can be triggered by removal of the fetuses, while the contralateral horn with fetuses remains large. Consistent with this observation, the empty horn in the pregnant cow remains small, regardless of the presence of developed placentomes with blood circulation from the pregnant horn.

The following observations suggest that activation of biochemical mechanisms for uterine involution may occur before delivery: (1) placentomes collected from the uterus at term (≥270 days) are relatively smaller than those collected at day 240; (2) dexamethasone administered before delivery, instead of immediately after delivery, can induce RFM; (3) injection of relaxin (collagenase inducer) after delivery cancels the RFM-inducing property of dexamethasone injection; (4) softening (collagen breakdown) of the cervix occurs before delivery; and (5) plasma collagenase activity increases at term, but before labor. It is suspected that collagenase is activated before delivery about 9 hours before the release of placenta in the cow.

Failure of Cotyledon-Caruncle Detaching Mechanisms

It is proposed that the biochemical disturbance leading to RFM may be triggered either before or during delivery. The collagenolytic activity of cotyledon villi is decreased in cows with RFM, and persistence of type III collagen is observed in cows with RFM. Activity of matrix metalloproteinase-9 (MMP-9) in cows with retained placenta is lower than in cows without retained placenta, and specific active forms of MMP-2 are absent in retained placenta. The differences in enzyme activity between cows with and those without retained placenta may affect the hydrolysis of collagen and subsequent release of the

fetal membranes.[2] These observations suggest that a deficiency of collagenase may be involved in the hydrolysis of type III collagen. It is possible that RFM also may be due, in some cases, to the presence of an anticollagenase system in the placenta, because intraplacentome injections of collagenase are unable to hydrolyze collagen in 15% of cows with RFM. Collagenases are calcium-dependent enzymes; however, the decreased level of serum calcium found in cows with RFM does not preclude collagenase activity. Several factors have been related to failure of cotyledon-caruncle detachment.

Hormone Imbalances

Hormone imbalances existing before delivery are effective in inducing RFM (high-risk factor; see Table 45-4). Progesterone, more than estrogen, inhibits uterine collagenases and slows uterine involution. Dexamethasone increases synthesis and utilization of progesterone by cotyledon tissues in the cow. These changes may contribute to blocking postpartum expression of cotyledon collagenases. Moreover, it has been found that glucocorticoids down-regulate collagenases. Because of inconsistent reports, the mechanism for dexamethasone and prostaglandin F_2 alpha ($PGF_{2\alpha}$) to induce delivery with a high incidence of RFM remains unclear. It has been proposed that increases in prepartal $PGF_{2\alpha}$ metabolites and cortisol may constitute an indicator of RFM in cows. In one study, bovine production of $PGF_{2\alpha}$ by cotyledons predominated when membranes were released, whereas prostaglandin E_2 (PGE_2) production predominated if fetal membranes were retained. In the rabbit, however, PGE_2 and $PGF_{2\alpha}$ increased collagenase activity. The fact that previous retention is a significant risk factor (see Table 45-4) suggests that RFM may be due in part to a random expression of a malfunctioning gene that regulates uterine involution, including cotyledon proteolysis. Gene expression of proteolytic enzymes can be modulated by steroidal hormones; however, the prepartal hormonal signal to block cotyledon proteolysis and cause RFM has not been identified. Some critical hormone imbalances leading to RFM are presented in Table 45-4.

Leukocytes and Chemotaxis

The role of leukocytes and chemotaxis in the etiology of RFM has been widely discussed. Deficient neutrophil phagocytic activity, decreased migration, and decreased superoxide anion production have been proposed as factors in the pathogenesis of RFM in cattle. In fact, circulating neutrophils from cows with RFM produced less superoxide anion than did neutrophils from control cows. Positive chemotaxis resulted in an RFM incidence of 2.6%, and negative chemotaxis, 35.6%. Moreover, leukocytes are a mobile source of collagenases and may be involved in uterine regression and release of placenta.

Major Histocompatibility Complex

It has been proposed that major histocompatibility complex (MHC) provides an initial trigger for expulsion of the placenta and that consequently, maternal tolerance of fetal MHC products will lead to RFM, this mediated by temporary immunodeficiency.

Integrative Mechanism of Placenta Attachment/Detachment

Serotonin is suspected to be involved in placenta attachment/detachment and in various aspects of pregnancy and parturition in cows. Blood concentrations of serotonin are extremely high (54 μM) in the fetal calf during pregnancy and dramatically decrease (13 μM) at parturition to near adult cow concentrations. Coincident with decreased blood serotonin concentrations is decreased serotonin concentration in fetal membranes (cotyledon) during placenta detachment.[3] Thus, a physiologic pattern of serotonin concentrations related to placenta detachment during parturition exists in blood and placental tissues. Several important effects of serotonin have been recognized: (1) it has a proliferative effect on numerous cell types, including cultured bovine placentome cells, which may favor placenta attachment; (2) it inhibits secretion of proteolytic enzymes in bovine placentome cells, which may prevent placenta detachment during pregnancy; (3) it affects cortisol secretion, which may trigger parturition; (4) it is a powerful narcotic in newborn calves and probably in fetal calves, which may keep the fetus asleep during pregnancy. These four effects of serotonin can support a pivotal role for serotonin in placenta detachment, activation of the fetal-adrenal axis, and fetal awakening after prolonged gestational narcosis. The integrative hypothesis developed in our laboratory is presented in Figure 45-4.

Retained Fetal Membranes as a "Living" Organ

The lack of blood circulation to retained membranes and their release of offending odors suggest that these membranes are dead or nearly dead tissues. When membranes are kept in controlled laboratory conditions, however, they are capable of active utilization of oxygen and glucose, and more than 30% of the cells can exclude vital dye for 3 or more days. When membranes are kept at 1° to 2°C, they stay metabolically active for 8 weeks or longer. Fetal membranes have an outstanding potential to "survive" without a live fetus. These properties suggest that RFM may respond to ischemia, anoxia, and bacteria by releasing biochemicals that cause inflammation, thus predisposing the cow to metritis. It is suspected that chemical irritation of RFM by the intrauterine infusion of chemicals such as tetracycline may cause an inflammatory reaction that may cancel the beneficial effects of therapy.

Pathophysiology of Retained Fetal Membranes

A proposed flow chart for the pathophysiology of RFM is shown in Figure 45-5. Biochemical disturbances that inhibit cotyledon proteolysis may occur before parturition in the absence of uterine trauma and during parturition when uterine trauma is incurred. Prepartum inhibition of the cotyledon proteolysis mechanism is expressed after delivery by a lack of both cotyledon dehiscence and liquefaction of cotyledon-caruncle

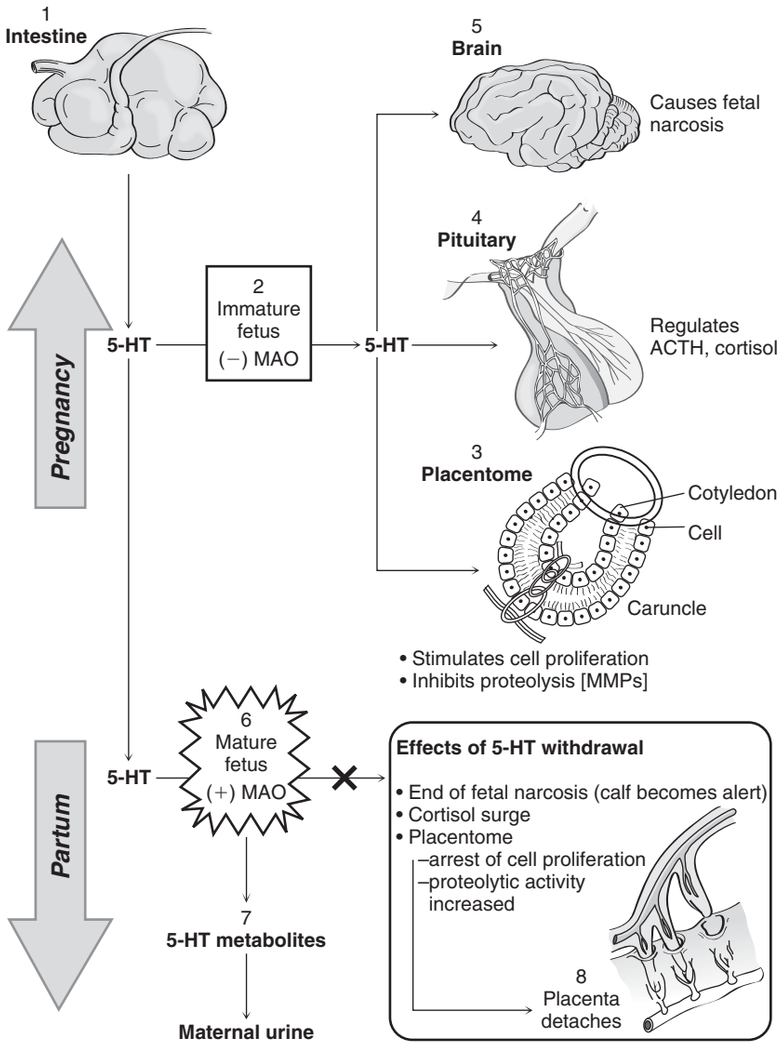

Fig. 45-4 Pivotal role of 5-hydroxytryptamine (5-HT, serotonin) in pregnancy, placenta detachment, and fetal narcosis. Integrative hypothesis. The main source (95%) of 5-HT is the intestinal wall *(1)*.[8] During fetal life *(2),* 5-HT may freely circulate through an immature fetal lung, liver, and placenta without significant inactivation, reaching high concentrations in the blood of fetal calves (6 times neonatal concentration),[3] humans, and goats. [9,10,11] In the placentome *(3),* 5-HT is a "growth factor" to stimulate placental cell proliferation[3] and inhibit placental matrix metalloproteinase activity,[12] which protect the "links" that favor placenta attachment.[13] Five-HT is a potent stimulator of pituitary ACTH secretion *(4)* in calves[14] and other species.[15,16] However, chronically high concentration of circulating 5-HT in fetal blood is hypothesized to suppress ACTH and cortisol secretions by down-regulation; this is to be investigated further. Moreover, excess of circulating 5-HT in the fetus may affect the brain *(5)* and cause fetal narcosis.[14,17] Fetal narcosis is a protective mechanism for the mother as well the fetus and may aid in keeping the placenta attached during pregnancy. Three days prior to partum, lung, liver, and placenta monoamine oxidase (MAO) enzyme systems (responsible for 5-HT inactivation) mature in rats, *(6)*[18] along with a transitory decrease of 5-HT content in the wall of the fetal intestine in calves.[19] Moreover, at the end of gestation there is an increased 5-HT uptake by the lungs in sheep.[20] The increase in MAO activity and 5-HT uptake may cause, in part, a drop in circulating 5-HT in the fetus. Increased MAO activity in the fetus may be the cause for more 5-HT metabolite to be excreted in maternal urine at the end of pregnancy in women.[10] A drop in circulating fetal 5-HT is proposed to reverse the physiological effects *(3–5)* of 5-HT during pregnancy, promoting cortisol surge in the fetus, placenta detachment, and alertness and walking behavior in the neonate *(8).* Experimental evidence using different animal species is available for most aspects of this hypothesis, however further research is needed to validate the above model for placenta detachment in bovines.

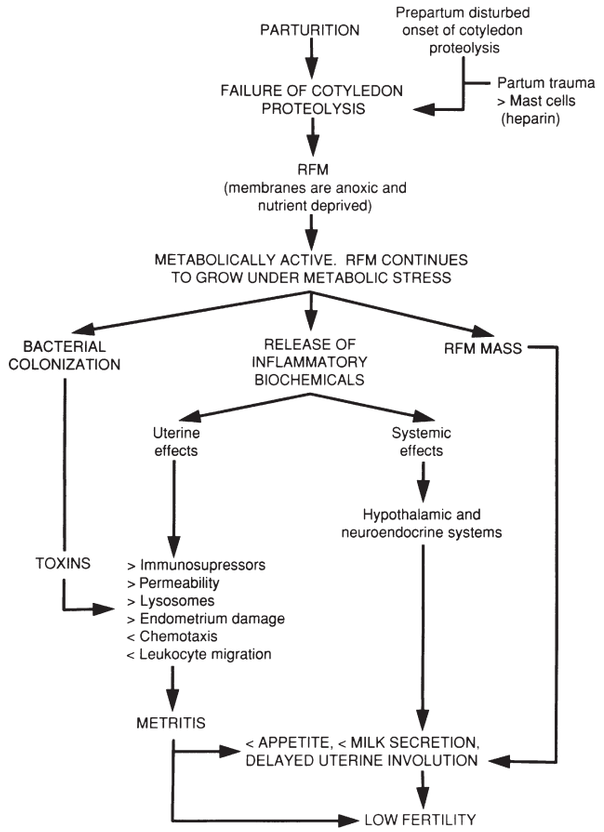

Fig. 45-5 Pathophysiology of retained fetal membranes. See text for explanation. (Copyright 2002 The University of Tennessee, College of Veterinary Medicine.)

interface–adhesive fluids. The retained membranes are ischemic, anoxic, and nutrient-deprived; nevertheless, they continue to grow and are metabolically active for several days. Under metabolic stress, retained membranes release inflammatory biochemicals that, in the uterus, cause immunosuppression (PGE_2), increased vascular permeability (histamine, prostaglandins), increased lysosome activity (proteolysis), endometrial damage (including release of heparin by mast cells), and decreased chemotaxis and leukocyte migration that lead to metritis and decreased fertility. Inflammatory biochemicals from RFM may cause systemic effects that are mediated by hypothalamic centers, including control of hormones, causing decreased appetite and milk secretion and possibly delayed uterine involution. Bacterial colonization is increased, favoring the release of both bacterial endotoxins and inflammatory biochemicals by RFM. Physical mass of the retained membranes (approximately 3–4 kg) within the uterus may contribute to delayed uterine involution, leading to the clinical complications and reduced reproductive performance associated with RFM.

Uterine trauma during calving has high risk factor values for RFM (see Table 45-4). One possible explanation for this association may be the release of heparin by an increased number of mast cells at the site of injury, causing the inhibition of both collagenases and collagen breakdown and delayed uterine involution. Inhibition of collagenase in placentome tissues may contribute to the occurrence of RFM.

RETAINED FETAL MEMBRANES AND METRITIS

RFM and metritis are positively correlated (see Table 45-2). In one study, 44% of lactations that began with RFM were eventually complicated by metritis. By contrast, only 16% of the lactations without RFM were complicated by metritis. The relative risk in that study for development of metritis in cows with RFM is therefore 2.8, and the attributable risk is 28%. A survey found that cows with RFM had a significantly higher incidence of metritis (53%) than cows without RFM (30%); also, a significant difference was found between conception rates in cows with RFM and metritis (66%) and in those with only metritis (77%). In an earlier study, metritis, not RFM, impaired reproductive performance; cows experiencing both RFM and metritis were more severely affected than cows that had only RFM or metritis.

Uterine involution, even in the absence of clinical complications, is a septic process; moreover, bacterial colonization increases during metritis. It has been proposed that the metritis that accompanies RFM results from the presence of decomposing placental tissues, which provide a favorable environment for bacterial colonization. Coliform bacteria and high concentrations of endotoxins present in lochia of cows with RFM are potent inducers of prostaglandins and cytokines, favoring development of uterine infections.[4] Bacteria found in the early postpartum uterus or their endotoxins may interact with retained membranes to secrete PGE_2, which may further predispose the uterus to infection (see Fig. 45-5).

RETAINED FETAL MEMBRANES AND MASTITIS

Although the main economic impact of RFM seems to be decreased milk production (more days open, decreased milk volume, milk from treated cows withheld), the correlation between RFM and mastitis is controversial (see Table 45-2). In a hospital-based study, cows with RFM (25.8%) were three times more likely to develop mastitis during hospitalization than cows without RFM (8.2%). In a different study, the relative risk factor for cows with less than 5 days' retention was 1.5, and 5.4 if retention lasted more than 6 days. More milk and more fat were recorded in cows with RFM than in cows without RFM in one trial, whereas in another, no correlation between RFM and mastitis was found.

TREATMENT

Objectives

The treatment objectives for RFM are to cause early detachment of the membranes in order to reduce the occurrence of metritis, decrease milk losses, reduce reproductive inefficiency, and decrease veterinary expenses.

Treatment versus No Treatment

Untreated cows more often are affected by endometritis and require repeat breeding than are treated cows. In a trial, uncomplicated retention did not affect the fertility of cows mated beyond 60 days of the last calving. In other studies, however, retention for more than 12 hours has been found to be detrimental to reproductive performance and milk production. Many clinicians believe that dairy cows with RFM should be treated, but the evidence regarding the advantage of treating RFM in beef cows is not conclusive.

Historical Overview

Many approaches have been used to detach retained membranes. These include manual removal, attachment of a weight to the membranes to speed expulsion, electrical stimulation of the membranes with a pulse generator to initiate uterine motility, acupuncture to dilate the cervix, and administration of uterokinetic drugs, sulfonamides, prostaglandins, antibiotics, antiseptics, and hormones. None of these methods is effective in the treatment of RFM.

Manual Removal

Manual removal of retained membranes is contraindicated because uterine infections are more frequent and more severe after this form of intervention than they are in untreated cows. Manual removal has been found to prolong the interval from calving to first functional corpus luteum by 20 days. When the placenta is not removed completely, a prolonged vaginal discharge follows. It is not easy to properly remove a retained placenta; 62% can be removed completely, 27% partially, and 11% are nonremovable. Often, attempts at removal during the first 48 hours after calving are unsuccessful because the placenta is too firmly attached and the apical part of the gravid horn is beyond the reach of the veterinarian.

Hormones

Hormones are not effective in either detaching the placenta in primary RFM or preventing early postpartum metritis in cows with RFM. Injections of relaxin have been shown to prevent the occurrence of RFM when dexamethasone is used to induce parturition. Clinical use of relaxin for either treatment or prevention of RFM has not been reported, however.

Prostaglandin

Use of $PGF_{2\alpha}$ in the treatment of RFM is of limited value. $PGF_{2\alpha}$ does not cause detachment of retained membranes, significant uterokinesis in the early postpartum cow, or improvement of reproductive performance.

Oxytocin

Oxytocin is the uterokinetic hormone of choice in the early postpartum cow. In one study, 200 U given intramuscularly (IM) caused an almost immediate uterokinetic effect that lasted over 2 hours, with no spastic contractions. In another study, however, large doses of oxytocin were shown to create uterine spasm. Therefore, doses of 20 U three to four times daily have been used. The value of oxytocin in the membrane detachment in primary RFM is questionable, because uterine motility is normal or increased in cows with primary RFM.

Antibiotics

Intrauterine and systemic antibiotics do not hasten detachment of retained membranes. On the contrary, inactivation of collagenase by tetracycline in some tissues has been reported. Bacterial collagenase and oxytetracycline, injected into the umbilical arteries, are compatible substances when combined as a treatment for RFM, however. One opinion is that antibiotics should not be used to treat RFM because they may delay the release of retained membranes by inhibiting the putrefaction process. Regardless of the undisputed benefit of antibiotics in the management of most kinds of bacterial infections, the value of antibiotics in the prevention and treatment of RFM metritis remains controversial. Single intrauterine applications of antibiotics often fail to prevent clinical metritis or to improve fertility in treated cows compared with untreated cows.

In one study, despite intrauterine treatment with 5 g of tetracycline powder on day 1 and 10.5 million U of procaine penicillin G IM on days 1, 2, and 3, 76% of the cows developed metritis. The reason for the lack of effectiveness of antibiotics has been attributed to the use of inadequate dosages, because it is difficult to attain adequate tissue concentrations of antibiotics in the uterus by using uterine infusions. Moreover, intrauterine tetracycline has been reported to have a negative effect on subsequent fertility. A possible explanation for this effect is that tetracycline may cause irritation of the endometrium and perhaps also of the metabolically active retained membranes. Chemical inflammation may override the beneficial effects of the antibiotic. Another complicating factor with infusion of antibiotics into the uterus is the extreme pH of the antibiotic solutions used, which may contribute to damage of the uterus. For example, tetracycline solutions can be strongly acidic (with a pH of 1.8–3.0) or alkaline (for oxytetracycline for IV injections, pH is 8.0–8.5). Nevertheless, there is consistent support for the use of antibiotics, especially in severe cases of metritis, provided that adequate dosage and route of administration are used. Umbilical arteries are a potential route to be explored in the treatment of RFM not only for antibiotics but for other drugs. The small mass of the retained membranes (3.0 kg for the purpose of dose calculation) and the lack of blood circulation favor a long-lasting effect of a single and relatively small dose of antibiotic. Under these conditions, the passage of antibiotic into blood and milk is unlikely. More research is needed in this area, however.

Antiseptics

A variety of antiseptics, including chlorhexidine and dilute iodine, have been used in the treatment and

prevention of RFM. In most cases, their efficacy remains to be demonstrated. These compounds should be used with caution, especially iodine preparations, some of which can be extremely irritating.

Collagenase

A new approach for the treatment of RFM is the injection of collagenase into the umbilical arteries retrieved from RFM. This approach may be superior to traditional treatments because it is specifically directed at correction of the lack of cotyledon proteolysis. Bacterial collagenase from *Clostridium histolyticum* is used because it can degrade several types of collagen, it is commercially available, it is affordable, and it does not cause residual blood clotting in placenta.

The technique. In this procedure, the umbilical cord is located and is recognized by two firm arteries and two veins (pencil diameter) that slip off the fingers when palpated. Once the cord is located, a second hand is introduced into the vagina and the cord is retracted by alternating hands in the vagina. Once the umbilical cord is in the vulva, the arteries are clamped with Kelly forceps. Collagenase solution* (200,000 U, plus 40 mg calcium chloride and 40 mg sodium bicarbonate dissolved in 1 L saline) is injected rapidly. Injection of collagenase solution can be accomplished easily by using a hand-pressurized 1000-ml saline bag attached to an intravenous administration set. The needle tip of the set is directly inserted into the artery (and ligated) without using a catheter. To ensure perfusion of the entire placenta, a volume of 1 L is injected. Oxytetracycline (100 mg total dose, which is approximately 30 mg/kg fetal membranes) for intravenous injection can be added to 1 L of collagenase solution if an antibiotic is desired; however, final pH of the solution should be adjusted to approximately 7.5. Five hundred ml of collagenase solution is injected into each artery, or 1000 ml into one artery if only one is available. Because of arterial anastomosis, it is unnecessary to inject the two arteries in single births or the two umbilical cords in twin births. Thirty-six hours later, the retained membranes are easily extracted by gentle traction if they have not been expelled spontaneously.

Comments. Collagenase treatment is effective in 85% of affected cows within 36 hours. In the 15% of cows that fail to respond, however, repeat treatment is not recommended because it rarely is effective. The treatment is safe and has no side effects. This therapy can be applied between 12 hours (best) and 96 hours after calving. After 48 hours of retention, there is a tendency for the residual blood in the placenta to clot and for anastomoses to close, which limits the perfusion of retained membranes with collagenase solution. The technique is simple, and the procedure can be completed in 25 minutes by a skilled veterinarian without an assistant. In approximately 6% of cows, sufficient retraction of the umbilical cord is not possible. The most time-consuming steps are retracting the umbilical cord and injecting 1 L of solution into the umbilical arteries. Another advantage of the treatment is that collagenase causes liquefaction of fetal membranes, and siphoning of uterine fluid is facilitated in cows treated with collagenase.

Hypothetically, oxytetracycline plus collagenase constitutes a useful, therapeutic combination, owing to the advantage of loosening retained membranes while preventing infection. Antibiotic-collagenase combinations should be used with caution, however, to avoid inactivation of collagenase by the antibiotic. Furthermore, infusions of collagenase into the lumen of the uterus are not effective.

Collagenase is not currently approved for use in food-producing animals in the United States. This procedure is highly effective in mares with retained placenta,[5] in which location of the large umbilical vessels for collagenase injection is easier than in cows. Moreover, placental collagen is more sensitive to collagenase hydrolysis in the mare than in the cow (and women).[6]

Collagenase and Cesarean Section

Cesarean section is followed by an increased incidence of RFM in cows (see Table 45-4). Injection of collagenase into the umbilical arteries during cesarean section is potentially effective in preventing RFM. A small number of cows undergoing cesarean section in our hospital have been treated in this manner, with excellent results.

Treatment with Hyperosmotic Solutions

Delivered placentas contain osmoactive substances that cause considerable retention of water and increased tissue volume (by 50%) when immersed in saline. Injections of osmoactive solutions or expandable solutes into the placentome via the umbilical circulation may cause mechanical detachment of cotyledons from caruncles by increasing cotyledon volume. This finding provided the rationale for the treatment with hyperosmotic solutions. This approach, however, has been tested without satisfactory results in our laboratory.

Future Treatment

We believer that future treatment for RFM may be based on the development of an inexpensive injectable substance capable of triggering the activation of cotyledon proteases (collagenases). Serotonin injections were thought to be a potential treatment for detachment of RFM in the bovine because addition of serotonin to cell culture medium stimulated secretion of the proteolytic enzyme collagenase by cultured rat and human myometrial cells. Moreover, in one study in which a preparation containing serotonin and ergometrine (uterotonic substance) was injected in cows after calving, the incidence of RFM was 10%, compared with 38% observed in control cows injected with saline.[7] We have not been able to confirm the researchers' result, however. Experiments in our laboratory in which serotonin was injected repeatedly into cows either suffering from RFM or at high risk for RFM failed to detach fetal membranes or to prevent occurrence of RFM. Furthermore, serotonin failed to induce collagenase activity in isolated placentomes and

*Type XI, Sigma Chemical Co., St. Louis, MO.

inhibited collagenase secretion by cultured placentome cells, which suggests that serotonin injections may not be an effective treatment for RFM. On the contrary, serotonin is suspected to favor placenta attachment in the bovine (Fig. 45-4).

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NOTE: Many references listed in this edition are new to this chapter and were not listed in the first edition version. For specific references to various statements, please see first edition reference listing.

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CHAPTER 46

Metabolic and Nutritional Diseases of the Puerperal Period

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Metabolic adaptations associated with the transition from pregnancy to lactation take place in all cows, including those that are well fed. These metabolic changes may become exaggerated, however, when maternal supplies of glucose, protein, or calcium (Ca), or any combination thereof, are inadequate, thus leading to metabolic disease problems. A poor transition is evidenced by slow or cyclic feed intake, excessive body condition loss, and high prevalence of metabolic and infectious diseases during the puerperium, followed by poor lactational performance.[1] Milk production in dairy cattle with periparturient disease is differentiated from that in nonaffected cows by low first-test and peak milk production and altered composition, typically a high (>1.5) fat-to-protein ratio.[2] As previously characterized, perturbed transition also accounts for poor reproductive performance[3] and increased involuntary cull rate.[4,5]

This chapter describes commonly encountered metabolic and nutritional diseases associated with improper transition from pregnancy to lactation.

DISEASES OF ENERGY BALANCE

All dairy cows, and to a lesser extent beef cows, experience a period of negative energy balance immediately before and through 4 to 6 weeks after calving. A variety of metabolic, hormonal, homeorhetic, and environmental factors influence individual cow response to negative energy balance and associated health, productive, and reproductive consequences. Metabolic alterations associated with the transition from pregnancy to lactation and their impact on substrate metabolism are described in Chapter 57. A cow's inability to maintain homeostasis of key metabolites during the transition period is prerequisite to a variety of periparturient diseases. Severe or prolonged negative energy balance resulting in rapid and excessive lipid mobilization, coupled with perturbed nonesterified fatty acid (NEFA) metabolism, a consequence of an inability to maintain glucose homeostasis, manifests in a number of clinical and subclinical syndromes of ketosis and fatty liver infiltration. To some degree, hepatic lipidosis usually precedes and is concurrent with development of deranged fatty acid metabolism, but fatty infiltration can become excessive and become a clinical entity itself. Several excellent detailed reviews of negative energy balance and its relationship to the pathogenesis of ketosis and hepatic lipidosis are available.[6–10]

Elevated blood NEFA concentration is requisite to excessive hepatic NEFA uptake as absorption is directly proportional to concentration.[11] Metabolic fate of absorbed NEFAs in the liver is limited primarily to partial oxidation (ketogenesis) or esterification to triglycerides (lipogenesis). Complete oxidation in peroxisomes and triglyceride export as very-low-density lipoproteins (VLDLs) are other disposal pathways for NEFA but are limited options in ruminant animals. Availability of glucose coupled with current metabolic milieu determines the fate (lipogenesis or oxidation) of absorbed NEFAs in the liver.[12] **Ketosis** is characterized by an excessive accumulation of ketone bodies when glucose availability is limited.

Kronfeld[13] suggested differentiating ketosis syndromes on the basis of underlying pathogenesis. Spontaneous or **primary clinical ketosis** was defined as a disease syndrome independent of an initiating disease process. This is in contrast with **secondary ketosis**, in which the ketotic state is induced by a primary disease process further exacerbating negative energy balance. **Alimentary** (or dietary) **ketosis** is defined as a ketotic state resultant from consumption of excessive ketone bodies or their precursors within the diet. Ensiled feeds exhibiting a clostridial fermentation will contain an increased amount (>1%) of butyric acid as a proportion of total volatile fatty acids (VFAs). Butyric acid is converted to β-hydroxybutyrate (BHB) by the rumen epithelium, which can contribute significantly to ketone body production. **Subclinical ketosis** is defined as elevation of blood ketone concentration (>14 mg/dl) in the absence of clinical signs of ketosis.[14]

Glucose supply is determined by fractional rate of hepatic gluconeogenesis and glucogenic substrate availability, given that minimal dietary glucose is directly available as a result of ruminal fermentation. Holtenius and Holtenius[15] suggested further characterizing spontaneous clinical ketosis into types I and II, based on differences in metabolic derangements of glucose homeostasis. Cows unable to meet glucose demands of milk production exhibit the metabolic underpinnings of type I ketosis: gluconeogenesis is maximized but sufficient glucogenic substrate is lacking.[15] These cows are severely

hypoglycemic and have low insulin-to-glucagon ratios, thereby facilitating a catabolic state utilizing amino acids for gluconeogenesis. Absorbed NEFAs are metabolized almost exclusively to ketone bodies to provide alternative energy substrate for tissues in an effort to increase glucose availability and to minimize detrimental effects of excessive body protein catabolism. This metabolic description is consistent with classic spontaneous ketosis associated with high milk production in early lactation; accordingly, the term **peak lactation ketosis**[7,12] is used for this entity in this chapter.

Another ketotic syndrome, type II ketosis, occurs more immediately around parturition and is characterized by moderate hyperketonemia, greater hepatic fat infiltration, and moderate hypo- to normoglycemia.[7] Metabolically, neither gluconeogenesis or ketogenesis is maximally stimulated, and more NEFAs are directed to esterification in these cows. As fatty infiltration increases, gluconeogenic capacity is compromised, ultimately leading to reduced glucose availability and increased ketogenesis.[12] Holtenius and Holtenius[15] attributed type II ketosis to insulin resistance and impaired glucose utilization as a result of dry period overfeeding and hence equated the syndrome to type 2 diabetes mellitus. **Periparturient ketosis** is a descriptive term suggested for this type II ketosis syndrome,[12] to be differentiated from peak lactation ketosis.

Clinical Signs

Cows affected with primary ketosis will not present different from any other early-postpartum cow experiencing one or more typical periparturient diseases. Careful physical examination should be completed to determine presence of current disease. Progressive loss of appetite and milk production over a period of a few days is a typical presentation for peak lactation ketosis.[12,16] Milk production losses vary depending on when energy equilibrium relative to dietary intake is established but may become severe, exceeding a 50% reduction in previous production. Ironically, cows will select against grain consumption, further exacerbating their energy deficit and, in conjunction with reduced dry matter (DM) intake, will rapidly lose body weight and condition. Affected cows often have a rough appearance, look gaunt and "tucked up" in the abdomen, show moderate depression, and have a sweet acetone smell to their breath. Vital signs typically are normal, with decreased rumen motility and firm, dry feces of reduced volume. A small proportion of ketotic cows (10%) will present with neurologic signs including abnormal licking, head pressing, circling, and walking blind.[16,17] Nervous ketotic cows may periodically show hyperesthesia, ataxia, tremors, or tetany, which may be confused with other neurologic diseases. The mechanism responsible for neurologic signs is unknown, and an individual cow's clinical presentation is not associated with blood ketone concentration. Most commonly, cows present with peak lactation ketosis between 3 and 6 weeks post partum, a period characterized by rapidly increasing milk production and lagging DM intake.

Cows experiencing periparturient ketosis will present with clinical signs similar to those in cows with peak lactation ketosis, with the following differences. Time of presentation occurs earlier, typically 7 to 10 days relative to calving, and cows have higher (>3.75/5.0) body condition score (BCSs). By definition, cows with subclinical ketosis show no diagnostic clinical signs, but herds experiencing problems with energy balance and high prevalence of subclinical ketosis may show lower milk production, reproductive inefficiency, and higher than expected prevalence of left displaced abomasum. Subclinical ketosis occurs during the same time frame as for clinical ketosis and most often is seen at approximately 4 weeks post partum.[14]

Clinical signs in cows with fatty liver are similar to those in cows with periparturient ketosis, and fatty liver may represent a more severe form of this disease. Affected cows typically present with clinical manifestations around the time of calving and have excessive body condition at the time of dry-off.[18] At presentation their BCS may be slightly reduced. Clinical fatty liver disease is more common in older cows but can be seen in primiparous cows.[18,19] Severity of hepatic fatty infiltration will determine additional accompanying clinical signs and secondary metabolic and infectious disease processes.[10] Morbidity and mortality rates in cows experiencing concurrent periparturient diseases and reproductive inefficiency increase with degree of fatty infiltration. Cows with severe fatty infiltration (>10% wet weight) will be extremely anorectic and depressed and may show neurologic signs.[19] These cows also show very poor response to therapies targeted at concurrent diseases, possibly a result of impaired immune function[10,20] and hepatic gluconeogenesis[21] and ureogenesis.[22] Even with aggressive therapy, death and culling rates are greatly increased among clinically affected cows.[18,19]

Diagnosis (Individual and Herd)

Ketosis

Definitive diagnosis of ketosis in an individual cow is established by measuring excessive concentration of ketone bodies in blood, milk, or urine. Signalment, clinical signs, and a thorough physical examination will suggest the form of ketosis present. Acetoacetate, acetone, and BHB comprise the ketone bodies generated from partial fatty acid oxidation. Acetoacetate and acetone account for only 10% to 20% of total ketone bodies, but this proportion increases during ketosis as acetoacetate production is greatly increased. As a result of acetoacetate instability in harvested blood specimens, however, measurement of more stable BHB concentrations typically is used in diagnosing ketosis with blood samples. Total blood ketone or BHB concentration in excess of 30 or 25 mg/dl, respectively, generally is considered a threshold for clinical ketosis.[12] Ketone bodies are concentrated in urine at 2 to 4 times blood levels; thus, total urinary ketones often exceed 80 mg/dl.[12,17] By contrast, milk ketone concentration is 40% to 50% of blood concentration; therefore, milk total ketone concentrations of 10 mg/dl or greater are found in clinical ketosis cases. Cows with peak lactation ketosis generally have more profound ketonemia (blood ketone level >40 mg/dl) compared with those with periparturient ketosis.[12] Based on greater risk

association with clinical ketosis and displaced abomasum and reduced milk production, subclinical ketosis in cows was defined as blood BHB concentrations greater than 14 mg/dl (1400 µmol/L).[14] Milk acetoacetate or BHB concentrations greater than 1 mg/dl (100 µmol/L) are considered indicative of subclinical ketosis.[14]

To facilitate cow-side diagnosis, a number of semiquantitative commercial tests are available for estimation of ketone body concentration in milk or urine. Powder or test strips based on the nitroprusside reaction measure acetoacetate concentration, whereas a newer test kit (Ketolac-BHB or Keto-Test) measures milk BHB concentration. As a result of its concentrating property, evaluation of urine ketone concentration results in an overestimation of ketosis prevalence, whereas milk ketone concentration underestimates ketosis prevalence. Studies evaluating cow-side test sensitivity and specificity relative to blood BHB concentration suggest using a threshold value of 1 mg/dl (100 µmol/L) of either milk acetoacetate or BHB as a good indicator of subclinical ketosis.[14,23,24] More recent work suggests that using a time-restricted (5 sec) interpretation of urine ketones (Ketostix) can be as accurate as milk ketone evaluation.[25]

Fatty Liver

Definitive diagnosis of hepatic fatty infiltration is determined by direct measurement of triglyceride content from a liver biopsy sample. Hepatic triglyceride content can be determined quantitatively by chemical methods or semiquantitatively by histologic volume or flotation methods[18,19,26] (Table 46-1). Ultrasound examination holds some promise as a noninvasive method to categorize liver fat content.[27]

Table **46-1**

Methods for Determining Hepatic Triglyceride Content

	DEGREE OF HEPATIC TRIGLYCERIDE INFILTRATION			
Methodology	***None***	***Mild***	***Moderate***	***Severe***
Histologic, % volume	0–5	5–20	20–40	>40
Chemical, % wet weight	<1	1–5	5–10	>10
Chemical, % dry matter	<3	3–15	15–33	>33
	HEPATIC TRIGLYCERIDE CONTENT (% DM)			
Buoyancy in solution (specific gravity)	***<13***	***13–25***	***25–35***	***>35***
1.000 (distilled water)	Sink	Sink	Sink	Float
1.025 (3.18% $CuSO_4$)	Sink	Sink	Float	Float
1.055 (8.11% $CuSO_4$)	Sink	Float	Float	Float

Data from Herdt TH, Goeders L, Liesman JS, Emery RS: Test for estimation of bovine hepatic lipid content. *J Am Vet Med Assoc* 1983;182:953–955; Herdt TH: Fatty liver in dairy cows. *Vet Clin North Am Food Anim Pract* 1988;4:269–287; and Gerloff BJ, Herdt TH: Fatty liver in dairy cattle. In Howard JL, Smith RA (eds): *Current veterinary therapy 4: Food animal practice.* Philadelphia: WB Saunders, 1999, pp 230–233.

Various liver function tests have been used to evaluate degree of fatty infiltration relative to clinical severity of ketosis and hepatic lipidosis. A wide variety of serum biochemical tests were evaluated for their ability to predict severity of hepatic lipidosis and hepatic function. Nearly all tests were of questionable value diagnostically and prognostically, as a result of their nonspecificity for liver function and minimal association with degree of liver fat content.[18,19,28] Biochemical measures of aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), and total bilirubin were significantly increased with severe hepatic lipidosis; however, most such measures were of low specificity.[28] Cows with severe hepatic lipidosis also tend to have much lower serum total cholesterol concentrations (<70 mg/dl), reflecting a reduction in total circulating lipoproteins.[19] Although not specific for liver function, elevation of AST (>100 U/L) has been suggested as the best supportive evidence of hepatic fatty infiltration.[18,19,28]

Other supporting clinicopathologic findings consistent with ketosis and fatty liver include elevated NEFA concentration and hypoglycemia. Prepartal and postpartum NEFA concentrations exceeding 0.4 and 0.8 mEq/L, respectively, are indicative of negative energy balance of a magnitude that increases risk for ketosis and other periparturient diseases. Blood glucose concentrations less than 35 mg/dl often are associated with peak lactation ketosis, whereas cows with periparturient ketosis or fatty liver may have slightly higher concentrations.

Herd Diagnostics

More important than individual cow diagnosis is determination of herd risk for ketosis, fatty liver, or both. Herd diagnostics can be based on selective metabolic profiling and dairy production records evaluation. Serum NEFA concentrations ante partum and post partum can be used to evaluate risk for excessive negative energy balance. Serum samples are collected from a minimum of seven animals in the prefresh (–14 to –3 days) and postfresh (<30 days) groups. If more than two samples have NEFA concentrations exceeding 0.4 mEq/L (ante partum) or 0.7 mEq/L (post partum), then these groups are at higher risk for periparturient disease and subclinical ketosis.[23,29] Similarly, serum BHB concentration can be measured in selected early postpartum animals to evaluate risk of subclinical or clinical ketosis. If more than two samples out of 10^{23} or 12^{29} total samples with BHB concentration exceeding 14.5 mg/dl (1400 µmol/L) is suggestive of increased herd risk for subclinical ketosis.

Given the association between ketosis/fatty liver and lower milk production, herd records can be used to evaluate milk production in early lactation cows. Lower than expected first-test-day milk or low peak milk relative to overall herd production indicate potential transition cow problems. Lower 305-day mature equivalent (305ME) milk production for early-lactation than for later-lactation cows also indicates possible transition cow problems. Lower early-lactation milk production, however, is not specific for ketosis or fatty liver but reflects problems associated with most periparturient diseases.[2]

Alterations in milk composition, primarily fat as a result of increased lipid mobilization during negative energy balance, seem to better reflect risk for ketosis and

fatty liver. Milk fat content is elevated in clinical and subclinical ketotic cows,[14] a result of increased NEFA delivery to the mammary gland from fat mobilization. Herds in which milk fat content is elevated in 20% of the cows or greater (5% in Holsteins, 6% in Jerseys) on first test date are undergoing rapid fat mobilization and are at greater risk for ketosis. A better indicator of herd ketosis risk is protein-to-fat ratio. A milk crude protein–to–fat ratio of 0.75 (1.33 fat-to-protein ratio) on first test date in 40% or more cows suggests that the herd is at greater risk for ketosis.[23,30] Cows with a milk fat–to–crude protein ratio greater than 1.5 were 3.2 times more likely to have clinical ketosis.[2] Conversely, cows with ketosis before first test date were 4.4 times more likely to have a high fat-to-protein ratio (>1.5); however, these cows also were at greater risk for other periparturient diseases.[2]

Treatment

Fundamentally, ketosis treatment objectives of glucose replacement and reducing fat mobilization have not changed over the past 50 years.[31–33] Bolus intravenous glucose or dextrose (250 g; 500 ml of 50% solution) in conjunction with intramuscular glucocorticoid administration (0.03–0.04 mg/kg dexamethasone [Azium, Schering] or isoflupredone acetate [Predef 2X, Pfizer]) is surprisingly effective in many cases of clinical ketosis. Glucocorticoid therapy without glucose administration is less efficacious.[34] Initial therapy is followed by continued supplementation with glucogenic substrates and appetite stimulants.

Cows with periparturient ketosis are less responsive to bolus glucose administration than are cows with peak lactation ketosis, most likely because of confounding effects of fatty liver. Although peak lactation ketotic cows respond quickly to glucose therapy, relapses occur, often necessitating repeated therapy. Daily bolus glucose therapy may be needed over a 2- to 4-day period for full recovery. Hyperglycemia mediated by bolus glucose administration is short-lived but induces a marked decline in NEFA release[35] and ketone production[36] and stimulates insulin[36] release. Fructose and sorbitol have been advocated as alternative glucose substrates to prolong the hyperglycemic effect. These compounds are exclusively metabolized to glucose by the liver and not appreciably utilized by peripheral tissues. No therapeutic advantages of these sugars over glucose have been found.[16,33] Xylitol may hold some therapeutic advantage.[37]

An alternative to daily injections is continuous glucose infusion for difficult or refractory cases or for cows with periparturient ketosis and fatty liver. Infusion of 20 L of 2.5% glucose in normal saline over 24 hours is suggested.[17] Such intensive infusion therapy may not be practical in many situations but is of value in genetically superior animals and for treatment of more severe hepatic fatty infiltration. Urine ketones should be monitored for response, and blood glucose concentration should be monitored after therapy to detect possible rebound hypoglycemia.

Glucocorticoids stimulate tissue protein catabolism, generating amino acid substrates for gluconeogenesis, and redirect glucose away from tissue and mammary gland utilization. The combined effect results in reduced milk production and improved glucose kinetics. The milk reduction effect of glucocorticoids cancels out the milk stimulation effect of glucose administration, which may account for the improved clinical response with combination therapy. Use of multiple corticosteroid injections, however, probably should be avoided because of the negative immunosuppressive (dexamethasone) or K depletion (isoflupredone acetate) effects of these agents.

Insulin plays multiple regulatory roles in fat and glucose metabolism that would be beneficial in ketosis therapy. Insulin is a strong suppressor of adipose NEFA release and ketogenesis and facilitates peripheral tissue uptake of ketone bodies and glucose. Low doses of long-acting insulin every 24 to 48 hours, along with glucose infusion or glucocorticoid therapy, have been found to be effective.[17,33] Previously, 200 to 300 U of protamine zinc insulin every 48 hours was used, but this agent is no longer available. Human recombinant insulin (25 IU/100 kg of Ultralente) every 24 to 48 hours has been advocated as an alternative insulin source.[17] In one study, use of a slow-release insulin (0.14 IU/kg) showed promise for treatment of ketosis and fatty liver.[38] In this study, higher dosages (0.29 or 0.43 IU/kg) resulted in adverse hypoglycemia.

A variety of glucogenic substrates—namely, glycerol, propylene glycol, and propionate salts—can be provided orally as a drench or feed additive to complement glucose therapy and maintain substrate to support gluconeogenesis.[31,37,39–42] Propylene glycol (1,2-propanediol) is most commonly used at a typical dose of 225 to 400 g (8 to 14 oz) orally twice daily.[33] Excessive dosing of propylene glycol (>800 g) may have adverse effects on rumen function and neuromuscular activity.[33,37] Glycerol can be administered at 500 g twice daily (1 L/day).[43,44] Sodium (Na),[31] Ca,[39,40] or magnesium (Mg)[37] salts of propionic acid can provide glucogenic substrate, as well as readily available mineral. Dosage ranges from 50 to 250 g twice daily administered as a drench or applied to the feed. Caution must be taken to avoid oversupplementing mineral from these propionic acid salts. All of these glucogenic substrates are metabolized to glucose by the liver. In the face of liver dysfunction due to fatty infiltration, efficacy of these substrates is questionable.

Treatment approach to fatty liver should reflect severity of fatty infiltration and underlying etiologic disorder. Treatment objectives of establishing glucose homeostasis and minimizing lipid mobilization mimic those in ketosis. A paucity of studies exist defining efficacy of therapeutic protocols for fatty liver. Most promising is the potential therapeutic application of continuous glucagon infusion for treatment of moderate[45] or severe[46] fatty liver. Glucagon therapy also shows possible application to periparturient ketosis, because impaired gluconeogenesis is hypothesized as an underlying concern.[12] Fatty liver therapeutic protocols should undertake aggressive ketosis therapy coupled with good enteral nutritional support.

Beyond initial therapeutic procedures, cows with ketosis and especially fatty liver can benefit from various supportive therapy modalities. With various degrees of

inappetence, a common presentation, stimulation of appetite is critical. Often B-vitamin solutions, typically B_{12}, are administered to nonspecifically stimulate appetite. Offering fresh-cut grasses or a variety of feeds may help stimulate intake in some cows. If rumen function is compromised, rumen transfaunation may be required to stimulate intake. Force-feeding gruels to provide substrate for rumen microbes may be necessary in more severe cases of fatty liver. Gruels should contain sufficient fermentable fiber sources (beet or citrus pulp, wheat middlings), protein (alfalfa meal, soybean meal, distillers' grains) and fermentable energy sources, but in limited amounts to minimize acidosis potential. Complete commercial cow pellets often have been used to make gruels if individual feed ingredients are not readily available. Finally, because ketosis and fatty liver often are complicated by a number of secondary disorders, the overall treatment plan should include needed therapy to address these diseases.

Prevention

Preventive practices focused on reducing risk of ketosis and fatty liver must emphasize reduction of negative energy balance and resultant increased blood NEFA concentration. Collectively, three areas should be addressed relative to this objective: prepartal and postpartum dietary composition, DM intake, and hepatic metabolic processing of NEFA. Factors influencing and approaches to managing DM intake, as well as formulating balanced diets relative to preventing periparturient disease, are summarized later in the section "Key Control Points for Transition Success."

Emphasis has been placed on increasing dietary energy density in the immediate prepartal diet, compensating for decreasing DM intake, in an effort to minimize negative energy balance and predisposition to ketosis and fatty liver.[47–49] Minor and colleagues showed positive effects of increasing dietary content of nonstructural carbohydrate (NSC) (i.e., sugars and starches) on energy balance and liver fat infiltration.[50] When rumen-fermentable carbohydrates are increased, however, metabolizable protein supply also is increased, suggesting that energy and protein in ruminant diets are not independent. Cows fed a high-energy and high-protein prepartal diet had lower serum NEFA concentration, NEFA-to-cholesterol ratio, and fatty liver score compared with cows receiving high-energy, low-protein or low-energy, low-protein diets.[51] The association of high-energy, low-protein prepartal diets with hepatic lipidosis is consistent with the original case descriptions of fat cow syndrome.[52,53] Cows afflicted with either ketosis or hepatic lipidosis were found to have lower serum apoproteins associated with VLDL structures, suggesting an inability of the liver to export triglycerides.[54] Limited supply of methionine and lysine has been suggested to reduce VLDL production and predispose to hepatic lipidosis.[55,56] Protein content of the prepartal diet above National Research Council (NRC) recommendations has been associated with decreased ketosis incidence,[57,58] but positive metabolic response to protein has not been consistent across all studies.[48,59] In view of the multiplicity of roles amino acids play in glucose and lipid metabolism, potential exists for mediation of periparturient disease by manipulating metabolizable protein supply, but mechanisms are still not well understood. Part of the problem with animal response to diet may relate to confounding effects of BCS on dietary feeding strategies.[60,61]

Management practices related to feed intake and nutrient supply are equally important, if not more so, in preventing adverse responses to negative energy balance. Dry cows with obese body condition (BCS of 4 on a 5-point scale) are more predisposed to fatty liver[10] and ketosis.[12] BCS management must start in mid- to late lactation, in view of the inability to dramatically increase or decrease BCS during the dry period. Optimal range in dry cow BCSs is 3.0 to 3.75 (on a scale of 1 to 5), with a greater percentage of cows having 3.25 or 3.5 BCS. Proper feed bunk management should focus on ensuring appropriate feed quality, adequate feed availability (>21 hours/day), cow bunk space (30 inches per head), and water availability and quality. Grouping strategies, stocking density, and pen movements should consider cow social behaviors and the expression of agonistic interactions, often resulting in reduced feed intake with exacerbation of negative energy balance.[62] Suggested stocking density for free stall systems, based on observed DM intake effects, is less than 90%. Minimum suggested space per cow for free housing systems is 80, 100, and 120 square feet for far-off dry, close-up dry, and maternity cows, respectively. Additionally, environmental conditions of cold and heat stress need to be addressed. Behavioral and physical facility management issues related to transition cows recently have been reviewed.[62]

A number of dietary supplements and feed additives have been advocated to prevent ketosis and fatty liver. These supplements either act as lipotrophic agents (niacin, choline), helping the liver to process lipid better, or improve availability of glucogenic substrate (glycol, propionate, monensin sodium [Rumensin, Elanco]). Niacin (or nicotinic acid), fed at 6 g/day starting 2 weeks ante partum and 12 g/day at calving through 90 days in milk, has been shown to reduce ketosis incidence[63] and to increase milk production.[64] Not all niacin supplementation studies, however, have shown metabolic responses consistent with ketosis prevention.[50,65] Obese cows may respond more favorably to niacin supplementation, as a result of its antilipolytic activity. Niacin is degraded in the rumen,[64] which may limit efficacy with oral supplementation.

Choline, a methyl donor and constituent of phosphatidylcholine, facilitates hepatic lipid export by increasing VLDL formation. Similar to niacin, choline is readily degraded in the rumen, necessitating delivery in a rumen-protected form to be efficacious when administered orally.[66] Early studies feeding increasing amounts of rumen-protected choline (regimens ranged from 0 to 20 g choline/day) showed modest to no impact of choline on energy balance and liver triglyceride measures.[67,68] In a controlled fatty liver induction study, feeding 15 g choline/day during the induction phase reduced blood NEFA and liver triglyceride.[69] Feeding 15 g choline/day after induction of fatty liver tended to increase liver triglyceride clearance. Liver triglyceride was reduced and

glycogen content increased when choline was fed at 25 g/day in the prepartal period (21 days) and at 50 g/day after parturition (60 days).[70] Other recent preliminary studies found no response to choline supplementation, possibly attributable to lower BCSs and a lesser risk for negative energy balance.[71,72]

Supplying glucogenic substrates, such as glycol, propionate, or propylene glycol, immediately before or after parturition has been advocated as a preventive for negative energy balance disease problems.[37,73–75] Prepartal fat supplementation also has been advocated to improve transition energy balance,[76] but drenching fat did not show a positive response.[77] Administration of 1 L of propylene glycol or glycerol per day as a drench for 10 days resulted in reduced blood NEFA and BHB concentrations. Shorter-term drenching protocols (3 days after calving) did not decrease BHB concentrations.[74,75,77] In view of the labor-intensive nature of daily drenching, oral glucogenic precursor supplementation has been investigated. Oral drenching or bolus treatment (1 L or 1 kg/day) of propylene glycol[78] or glycerol[79] was more effective in reducing blood NEFA concentration, a result of increased insulin secretion, than was feeding the glucogenic precursor incorporated into a total mixed ration (TMR). Feeding 250 g of dry propylene glycol (60%) per day for 3 weeks after calving tended to reduce blood BHB but not NEFA concentrations.[80] In this study, the response to propylene glycol in the TMR may have been confounded by the greater DM intake for this treatment group. DeFrain and associates[81] fed 1 kg glycerol in a TMR and observed a decline in DM intake; nevertheless, a modest effect on postpartum NEFA concentration was noted. Metabolic response to dietary incorporation of glucogenic precursors is promising relative to a practical control of ketosis.

Canadian research has shown supplementation of a controlled-release monensin capsule 21 days ante partum to be effective in reducing diseases associated with negative energy balance and improving cow health and production.[82–85] Most recently, monensin sodium (Rumensin, Elanco) has been approved in the United States as a feed additive for lactating dairy cows. Although monensin was approved as a feed additive to improve feed-to-milk efficiency, its effect on rumen microbial fermentation to increase propionate production may help stabilize glucose homeostasis in transition cows and be beneficial in preventing ketosis.[86] In agreement with studies using the controlled release capsule, daily prepartal monensin supplementation has been shown to reduce blood NEFA and BHB concentrations and to improve glucose kinetics and consequently has potential for ketosis prevention.[87–89]

In addition to implementing various targeted dietary and management preventive strategies, application of a herd ketosis monitoring program (refer to "Herd Diagnostics" section earlier) is warranted and cost effective.[23,29,90,91] Monitoring prepartal NEFA concentration will highlight risk potential for fatty liver, ketosis, and other periparturient diseases. Monitoring postpartum BHB concentration will more specifically target subclinical or clinical ketosis risk, as well as related risk for left displacement of the abomasum (LDA).[23,92,93]

DISTURBANCES OF MACROMINERAL HOMEOSTASIS

Although most often associated with skeletal mineral and blood electrolytes, macrominerals have many important metabolic roles. Most macrominerals are homeostatically regulated to various degrees, and this process often becomes perturbed around the time of calving, resulting in clinical signs. Primary disease syndromes, which commonly manifest with muscular weakness and recumbency, have been described for calcium (Ca), magnesium (Mg), phosphorus (P), and potassium (K) deficiency. Although critically low blood mineral concentration is a diagnostic hallmark of these diseases, elucidated mechanisms suggest that interactions among dietary macrominerals play some role in the disease process, especially for Ca and Mg deficiencies.

Hypocalcemia

Parturient hypocalcemia (milk fever, parturient paresis) is an acute Ca deficiency condition that manifests with progressive loss of skeletal, cardiac, and smooth muscle function. The disease most commonly occurs in high-producing adult cattle within 48 hours of calving, although a small percentage of cases occur ante partum and between 48 and 72 hours after calving. It is the most common metabolic disease of dairy cattle but also occurs in beef cattle infrequently. Parturient hypocalcemia is problematic as a result of its association with eight other periparturient disease processes and its negative effect on postpartum DM intake.[94] Costs associated with a clinical hypocalcemic event were estimated at $334 per case. Overall incidence of clinical hypocalcemia is approximated at 8% to 9%,[95] but variation between herds is tremendous, ranging from none to greater than 60% prevalence.[96]

Subclinical hypocalcemia is defined as total serum Ca concentration below 8.0 mg/dl in the absence of clinical signs of hypocalcemia.[97] Cows with subclinical hypocalcemia are thought to be predisposed to lower intake and other secondary diseases, including LDA.[98] Cows with subclinical hypocalcemia had higher NEFA concentrations compared with normocalcemic cows.[97] Cows experiencing one or more postpartum disease events had lower total Ca concentrations in the prepartal period (8.1 versus 8.5 mg/dl) and post partum (8.1 versus 8.9 mg/dl) compared with healthy cows.[99] When total serum Ca concentration before or after calving was below 8.0 mg/dl, cows were 1.6 times more likely to experience postpartum disease.[99] Using samples collected during the 2002 National Animal Health Monitoring dairy study, subclinical hypocalcemia was found in 25.3%, 43.9%, and 57.8% of samples collected (within 48 hours of calving) from first-, second-, and third- (and greater) lactation cows, respectively.[97]

Pathophysiology

In calcium homeostasis and mechanisms inducing hypocalcemia, the underlying pathogenesis has been related to a failure of the Ca homeostatic system to maintain blood Ca^{2+} concentrations with the onset of lacta-

tion.[100] Parathormone (PTH)—which responds to low blood Ca—and calcitonin (CT)—which responds to high blood Ca—are counterregulatory hormones precisely controlling blood Ca concentration within a narrow physiologic range. PTH, through its action on the kidney, hydroxylates circulating 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol (1,25-DHCC), the biologically active form of vitamin D. Kidney, intestine, and bone—primary target organs for PTH and 1,25-DHCC to increase Ca entry into the plasma pool—appear to be unresponsive to calcitropic stimulation. The acute loss of Ca in colostrum and milk at calving is not ameliorated by influx from intestine or bone, and hypocalcemia develops. Pool size of labile bone Ca, as well as number of vitamin D receptors, decreases with age, accounting for observed greater hypocalcemia prevalence in older cows.[101] Similarly, Jersey cattle have fewer vitamin D receptors compared with Holstein cows,[102] possibly accounting for greater hypocalcemia prevalence in Jersey cattle.

Goff[103] has reviewed specific factors that have been implicated in altering target organ response leading to hypocalcemia. Beyond age effects on receptor numbers, responsiveness of target organs can be suppressed by hypomagnesemia, hyperphosphatemia, and acid-base status resultant from dietary alterations. Hypomagnesemia impairs release of PTH and the interaction between PTH and its target organ receptor. Hyperphosphatemia interferes with activation of vitamin D by inhibiting activity of renal 1-hydroxylase enzyme. Dietary cation-anion difference (DCAD), as defined by the formula (Na + K) − (Cl + S), influences blood acid-base status and ultimately Ca homeostasis. Feeding a diet relatively higher in cations than in anions induces a metabolic alkalizing effect that is believed to blunt target tissue response to PTH stimulation. Acidification, achieved by feeding a diet relatively higher in anions than in cations, induces a release of bone mineral, primarily Ca, in an effort to support blood buffering systems. This released bone Ca is lost through the kidneys but can be reabsorbed under appropriate stimulation during a hypocalcemic episode. Forage-based diets often are high in K content and greatly contribute to excessively high-DCAD dietary conditions. Sodium-based feed supplements, namely, calcium bicarbonate, also can contribute to excessive cation intake and initiate an alkalizing situation leading to dysregulation of Ca homeostasis.

Clinical Signs

Clinical signs associated with parturient hypocalcemia occur as a spectrum of progressive muscular weakness. Progression of clinical signs often is characterized into three clinical stages based on serum concentration of ionized Ca^{2+} (Table 46-2).[96,104] Association between ionized Ca and muscle function is a result of the multiple roles Ca plays in nerve conduction, neuromuscular stimulation and muscle fibril contractility.[96,104] Mild hypocalcemia results in destabilization of peripheral nerve membranes, which may account for observed hyperexcitability and fine muscle tremors. Ca facilitates movement of actin and myosin fibers during contractility and release of acetylcholine at the myoneural junction. Progressive hypocalcemia could result in decreased acetylcholine release and muscle contractility, accounting for the ensuing flaccid paralysis observed in more severe cases.

Diagnosis

Determination of low plasma or serum Ca concentration is diagnostic. Practical ability to timely measure blood Ca

Table **46-2**

Clinical Presentation of Hypocalcemic Cows Based on Blood Calcium (Ca) Concentration

Parameter	Stage 1	Stage 2	Stage 3
Serum total Ca*	5.5–7.7 mg/dl	4.0–6.0 mg/dl	<4.0 mg/dl
Serum ionized Ca	2.75–3.85 mg/dl	2.0–3.0 mg/dl	<2.0 mg/dl
Posture	Standing but ataxic and wobbly, stiffness to hind limbs; reluctance to move	Sternal recumbency	Lateral recumbency; complete muscle flaccidity
Attitude	Alert with hypersensitivity and excitability	Alert to depressed	Depressed to comatose
Appetite	Diminished especially for grain	Anorexic	Anorexic
Temperature	Normal to slightly elevated	Low normal to subnormal, 96.4–100.4°F (36–38°C)	Subnormal, <98.6°F (<37°C)
Pulse	Normal, somewhat muffled	Slightly increased (<90 bpm) and weak	Rapid (>110 bpm), weak, muffled heart sounds
Other signs	Ear twitching and fine muscle tremors	Cold extremities (ears, legs); dry muzzle; constipation with dry feces; urine stasis—filled bladder	Possible bloat with aspiration pneumonia

*Influenced by serum albumin concentration and pH relative to association between clinical signs and total Ca concentration. Low albumin increases and alkalosis decreases ionized Ca concentration, altering the association between presenting clinical signs and total Ca concentration.
bpm, beats per minute.

concentration before treatment is limited, however. History and presenting clinical signs, in conjunction with rapid response to Ca therapy, are more typically used in diagnosis.[96] Collection of a blood sample before therapy is encouraged because this sample can be analyzed later if response to therapy is unfavorable. In addition to hypocalcemia, clinical chemistry abnormalities observed in uncomplicated cases include hypophosphatemia (<3 mg/dl), hypermagnesemia (>3 mg/dl), and hyperglycemia (>100 mg/dl). Elevated PTH concentrations associated with hypocalcemia may account for observed hypophosphatemia, due to increased renal excretion, and hypermagnesemia, a result of increased renal reabsorption.[103] Elevated cortisol levels associated with parturition and other stress factors could account for observed hypoglycemia. Depending on duration and bedding conditions of a recumbent hypocalcemic cow, muscle enzymes, creatine kinase (CK), and aspartate transferase (AST) may be elevated. Other concurrent conditions, especially hepatic lipidosis, could alter blood specific liver function and energy balance analytes accordingly.

A thorough physical examination is critical in evaluating a recumbent cow. Many other disease conditions can mimic hypocalcemia or complicate cases. Considerations in the differential diagnosis include musculoskeletal (fractures, luxations, compartmental crush syndrome) and neurologic (nerve paralysis, calving paralysis) injuries, acute toxic infections (mastitis, metritis, peritonitis), metabolic derangements (hypomagnesemia, hypokalemia, ketosis/hepatic lipidosis), and hemorrhage.[96,104]

Treatment

Without appropriate timely treatment, hypocalcemia can be fatal in approximately 60% to 70% of cases. Notwithstanding, 6% of affected cows die during treatment. Therapy is directed at replacing and maintaining serum Ca concentration. Calcium solutions are cardiotoxic, so careful intravenous administration relative to rate and dosage is important. Calcium solutions should be administered intravenously to effect, while monitoring heart rate and rhythm and clinical response. As a general rule, Ca solutions should be dosed slightly less than 2 g Ca per 100 kg (220 lb) of body weight and at a rate of 1 g Ca per minute.[105] Underdosing is not effective and results in more relapses and increased risk for injuries from struggling to rise. Hypercalcemia is a concern not only from potential cardiotoxicity but for its suppressive effect on PTH release and delay in maintaining Ca homeostasis.[105]

Even though many different chemical forms of Ca can be administered effectively, most commercial Ca preparation solutions contain either calcium gluconate (9.3% Ca), a less caustic form compared with $CaCl_2$, or calcium borogluconate (8.3% Ca), a fairly soluble and stable solution. Typically, commercial Ca solutions contain between 8.5 and 11.5 g Ca per 500 ml. In spite of multiple blood chemistry abnormalities in hypocalcemic cows, therapeutic solutions need contain only Ca. Correction of hypocalcemia typically will correct other abnormalities.

Response to intravenous Ca therapy should be fairly immediate, with obvious changes in muscle function. Heart rate should return to normal, and heartbeat should increase in strength. Muscle fasciculations may become evident in the flank and spread throughout the body. As smooth muscle activity returns, the cow may eructate, defecate, and urinate. A large proportion (60%) of treated cows will rise soon after therapy, with another smaller proportion (15%) recovering within 2 hours. Cows should be reevaluated if they have not recovered within 8 to 12 hours.

Although cows may show favorable response to intravenous therapy, 25% to 30% of cases relapse within 24 to 48 hours.[104] Some cows may have a delayed response to vitamin D activation, resulting in cyclic relapses.[106] Other cows, as a result of high milk production, are unable to maintain Ca homeostasis. Calcium solutions can be administered subcutaneously, but the time required for correction from hyper- to normocalcemia is 4 to 5 hours, similar to that with intravenous therapy.[105] Less severe hypercalcemia after subcutaneous administration is observed. Subcutaneous administration can be associated with local injection site reactions as a result of Ca-induced cellular necrosis. Solutions containing dextrose also can be injurious as a result of their high osmolarity. To limit potential injection site lesions, solutions without dextrose should be used, and the volume limited to 50 or 75 ml (1 to 1.5 g Ca) per site.[105] The injection site is massaged to help distribute the bolus injected. Response to subcutaneous Ca administration is dependent on peripheral perfusion; even so, it is not as therapeutically effective as intravenous treatment. Caution is warranted before administering intravenous treatment to a cow that has been repeatedly treated subcutaneously without success, because the risk of cardiotoxicity from the combined doses is more likely.[96,105]

Oral supplementation of highly soluble Ca salts can augment parenteral therapy to treat mild hypocalcemia or minimize relapses.[107,108] Passive diffusion of ionized Ca across cellular tight junctions can occur when Ca concentration is sufficiently high.[105] A dosage of at least 50 to 75 g Ca is needed to drive the passive absorption process. Calcium chloride ($CaCl_2$) (36% Ca) often is used but is irritating and can induce metabolic acidosis. Physical injuries to the pharyngeal area can occur with use of tubed gel products, as well as chemical injury when gel tubes contain $CaCl_2$. Aspiration pneumonia can occur when the solution is provided as a drench. Calcium propionate (21.5% Ca) can be used similarly to $CaCl_2$ as an oral supplement. Use of calcium propionate may be preferred because it is less irritating, does not induce metabolic acidosis, and provides propionate as a glucose precursor. Supplying 250 or 350 g calcium propionate provides approximately 54 or 75 g Ca, respectively. Oral Ca supplementation can maintain elevated blood Ca concentration for 5 to 7 hours. Besides amount of Ca supplied, form (gel, slurry, drench) and volume will influence degree of passive Ca absorption and response.[105,108,109] Oral and subcutaneous Ca administration should be used prophylactically, in early stages, or for relapsing cases. Recumbent cows should receive appropriate intravenous therapy. Caution is advised in combining therapies, because the potential for cardiotoxicity is increased and stabilization of Ca homeostasis is delayed. Prevention of

hypocalcemia is discussed later under "Maintaining Calcium Homeostasis."

Hypophosphatemia

Phosphorus (P), as the phosphate anion (PO_4^{2-}), plays important biologic roles in skeletal mineral content, blood and rumen acid-base status, cellular energy transfer, metabolic reactions, and phosphorylated molecules (phospholipids, nucleic acids, phosphoproteins). Homeostatic regulation of blood P mimics that of Ca, sharing the same regulators PTH, CT, and 1,25-DHCC. Unique to P regulation is salivary recycling in support of rumen microbes, which accounts for 30 to 90 g P lost per day, or nearly 10 times the total extracellular P pool.[103]

Profound dietary P deficiency will lead to altered bone mineralization, resulting in rickets or osteomalacia in young and growing or aged animals, respectively. Hypophosphatemia sometimes is associated with postparturient hemoglobinuria, a condition characterized by red blood cell membrane fragility and lysis with release of hemoglobin pigment into blood and urine. P deficiency is thought to impair red blood cell energy production via glycolysis, thereby impeding the red blood cell's deformability as it passes through small-diameter capillaries.[110] Acute profound hypophosphatemia (<1 mg/dl) has been observed in late-pregnancy beef cattle and early-lactation dairy cows. Affected cows typically present with hindlimb weakness, inability to rise, bright alert attitude, and willingness to eat; such animals often are referred to as "alert downer cows" or "creeper cows." Increased P losses to fetal development or colostrum and milk production can precipitate acute hypophosphatemia, especially if the diet is marginal in P, although these situations often are further complicated by concurrent hypocalcemia, hypomagnesemia, and hypoglycemia.[103,111] In dairy cattle, acute hypophosphatemia occurs after calving, even with a P-adequate diet, and typically is associated with an unresponsive treatment for a hypocalcemic episode.

Hypophosphatemia (phosphate level of 1–2 mg/dl; normal range, 4–8 mg/dl) accompanies parturient hypocalcemia, but after appropriate Ca therapy, blood P concentration returns to normal. Increasing PTH in response to lower blood Ca concentration at parturition induces a decline in blood P concentration as a result of increased renal P clearance and salivary P recycling.[103,111] Intestinal P absorptive efficiency is influenced by 1,25-DHCC; however, a portion of recycled P is inherently lost in feces, resulting in a net loss of exchangeable pool P. In a small number of cows, recovery of blood P concentration after Ca therapy fails, culminating in a hypophosphatemic downer cow. The mechanism responsible for this lack of response has not been elucidated. Reduced rumen motility might impair recycled salivary P absorption.[103] Metzner and Klee observed low blood P concentrations in downer cows who failed to ruminate; however, hypophosphatemia was not considered a significant contributor to recumbency in their downer cow survey.[112]

Recumbent cows with hypophosphatemia (<2 mg/dl) can be treated with P supplements given intravenously, orally, or both. The chemical form of P has been shown to be important for biologic utilization and effective therapy where only the phosphate anion is biologically available. Derivatives of phosphinic acid (hypophosphite [PO_2^{3-}], phosphite [PO_3^{3-}]) are not biologically available.[113] Phosphite salts are more water soluble and do not precipitate in the presence of Ca or Mg, thus allowing their common use in commercial multiple electrolyte solutions. For intravenous treatment, 6 to 7 g P is the desired total dosage, which can be supplied by 23 to 30 g monosodium phosphate or one sodium phosphate–based human enema product (Fleet Enema).[105,113] The mineral source should be diluted in 1 L saline and the enema in 1 L water to reduce adverse effects from pH and tonicity, respectively. Calcium solutions should not be added to either of these preparations because they will induce precipitation of calcium phosphate. A more sustained blood P concentration with lower peak concentration after therapy is achieved with oral monosodium phosphate supplementation. A dose of 50 to 60 g P from 200 to 300 g monosodium phosphate (25% P) mineral is desired.[105] The mineral can be suspended in water and administered as a drench, or preferably through a stomach tube. Gelatin boluses or gels containing the appropriate amount of phosphate source also are therapeutic. Other phosphate mineral sources (dicalcium phosphate, bone meal) are not soluble and will not evoke a change in blood P as rapidly. Intravenous phosphate supplementation will maintain blood concentrations for only a couple of hours, compared with longer than 6 hours with oral supplementation.[105,113] With the mechanism responsible for inhibiting P recovery after hypocalcemia uncertain, the best method of preventing hypophosphatemia is to minimize risk of parturient hypocalcemia (see "Maintaining Calcium Homeostasis" section).

Hypomagnesemia

Magnesium is an essential divalent cation (Mg^{2+}), the second most abundant intracellular cation, and supports a multitude of metabolic processes as an enzyme activator. A large proportion of body Mg (65% to 70%) is located in bone mineral matrix, yet extracellular Mg (1% of total body Mg) is critically responsible for synaptic transmission between nerve and skeletal muscle.[114] Calcium homeostasis and Mg are closely intertwined. Both PTH and 1,25-DHCC production and secretion are adversely affected by hypomagnesemia, accounting for concurrent hypocalcemia in hypomagnesemic animals.[115,116] Magnesium also influences ability of PTH to interact with its cell receptor on target organs.[111]

Plasma Mg concentration (normal range, 1.8 to 3.0 mg/dl) is controlled primarily through dietary Mg concentration and absorption. Net renal excretion, under PTH control (which stimulates renal Mg conservation), attempts to maintain plasma concentration in the face of changing dietary levels.[114,115] Preruminant calves absorb dietary Mg efficiently from the small intestine (as is observed in nonruminant species), but functional ruminant animals absorb Mg predominantly from the rumen and reticulum. Rumen-soluble Mg can be absorbed via

an Na^+,K^+-ATPase transporter or by non–transporter-mediated paracellular movement.[111,114] Paracellular movement (passive diffusion) is facilitated by high dietary Mg (0.35% to 0.4% of DM) concentrations.[111]

Dietary Mg absorption is constrained by a number of dietary factors. High dietary K, nitrogen (N), Ca, and fat and low dietary Na are associated with reduced Mg absorption.[114,117] High dietary K increases ruminal K concentration, thus changing the K gradient and decreasing the electrical potential difference necessary to drive the Na^+,K^+-ATPase pump for Mg absorption.[114] Lush spring growth grasses typically are high in K and low in Mg and Na. Forages with a K/(Ca + Mg) ratio greater than 2.25 (values as mEq/kg of DM) are suggested to have greater risk for inducing hypomagnesemia tetany.[118] Lush spring grasses also contain large amounts of sucrose and organic acids, a primary one being *trans*-aconitic acid. *Selenomonas ruminantium,* one of the most prevalent rumen bacteria, preferentially ferments sucrose and has been shown to reduce *trans*-aconitate to tricarbayllic acid, an extremely potent divalent cation chelator.[119] Crested wheat grass and other range grasses can contain between 2% and 6% of DM as *trans*-aconitate.[119] This level of organic acids, in combination with low plant Mg content, may further contribute to higher risk potential for hypomagnesemic tetany under range conditions. Excessive dietary K is most responsible for interfering with Mg availability in dairy rations.

Loss of Mg with late fetal development or milk production, in association with reduced dietary Mg intake or availability, or both, can lead to reduced plasma Mg concentration with precipitation of clinical signs. Clinical syndromes of hypomagnesemia are named according to their inciting cause, such as grass tetany, lactation tetany, wheat pasture tetany, or winter tetany. Clinical signs and presentation are reflected by plasma Mg concentration. Moderate hypomagnesemia (plasma magnesium concentration of 1.1 to 1.8 mg/dl) is characterized by nervousness, low production, and low feed intake and often has an insidious onset. Cows with moderate hypomagnesemia may be more prone to parturient hypocalcemia.[105] Weather or other stressors that acutely reduce feed intake may precipitate manifestations of clinical tetany. At plasma Mg concentrations below 1.1 mg/dl, initial clinical signs include excitability; hyperesthesia; muscle fasciculations of face, shoulder, and flank; ear twitching; and ataxia resulting from leg spasticity during walking.[117,118] As plasma Mg concentration falls below 0.5 mg/dl, signs progress from staggering to recumbency with clonic convulsions and muscle rigidity. Opisthotonos, leg paddling, involuntary nystagmus, and seizures follow as the condition progresses, culminating in coma and death.

Tremendous individual variation occurs in associating clinical signs with plasma Mg concentration. Tetanic signs may be related more closely to cerebrospinal fluid Mg concentration (<1 mg/dl), which is stable over a wide range of plasma Mg concentrations but critically declines when plasma Mg is below 0.6 mg/dl.[114] Low serum Ca concentrations also may play an important part in manifestation of tetany.[105,117] Diagnosis is based on history and clinical presentation and confirmed by blood analysis for Mg and Ca. Postmortem vitreous humor Mg concentrations below 1.16 mg/dl are suggestive of hypomagnesemia as a cause of death.[117] Cerebrospinal fluid and vitreous humor Mg concentrations can be used diagnostically between 12 and 48 hours post mortem. Urine Mg concentration can also be used diagnostically as concentrations decline rapidly as hypomagnesemia increases.[118] Herd dietary Mg status can be assessed by determining serum Mg concentration in blood samples collected from multiple cows during the immediate (12 hours to 3 weeks) postpartum period. Goff suggested that at least 9 of 10 cows should have serum Mg concentration of 2.0 mg/dl or greater for dietary Mg status to be considered adequate.[111]

Treatment of clinical hypomagnesemia requires timely intravenous Ca and Mg salt administration with additional supportive therapy using subcutaneous and oral Mg supplementation to minimize relapses. Death will occur within hours if the disorder is not appropriately treated.[117] A dose of 2 to 3 g Mg provided as the chloride, borogluconate, or hypophosphite salt delivered slowly over 10 minutes is safe and effective.[105,111] Commercial preparations also contain Ca salts; therefore, slower intravenous administration is recommended to prevent cardiac dysrhythmias due to effects of Mg or Ca. Care must be taken in administering intravenous therapy because treatment may initiate a tetanic seizure, and if responsive, recovered animals may display extremely aggressive behavior and are dangerous. For this reason, and in the face of a large herd outbreak, adequate therapeutic response can be achieved by administering a magnesium chloride or magnesium sulfate enema (60 g in 200 to 300 ml of water).[117,118]

Relapse rate after intravenous treatment can be high and clinical response disappointing. Additional Mg delivered by subcutaneous injection of 50% magnesium sulfate solution (100 to 200 ml) can help minimize relapse rate through prolonged maintenance of serum Mg concentration. Oral therapy also can be used to increase rumen Mg concentration, facilitating passive diffusion to augment uptake; 100 g of magnesium oxide or 200 to 400 ml of 50% magnesium sulfate is administered as a drench.[105] A combination drench that includes Ca, P, and Na sources has been advocated to treat multiple possible deficiencies and to augment Mg uptake.[105,111]

Prevention of hypomagnesemia requires attention to dietary Mg content, addressing agronomic practices to minimize plant factors leading to decreased Mg content and availability, and strategic prophylactic Mg supplementation.[114,117,118] Dietary Mg concentration during the periparturient period should be maintained between 0.35% and 0.4% of DM. Either magnesium sulfate or oxide mineral can be used to augment dietary content. Mg-fortified (5% to 8% Mg) salt blocks can be supplied; however, intake is variable because of poor palatability. Typically these blocks contain molasses to aid palatability. Another option is to blend one-third magnesium oxide, one-third trace mineral salt, and one-third soybean meal or distillers' grains as a free choice supplement. Salt blocks also increase Na intake, which can facilitate Mg absorption. Agronomic and management practices include grazing mixed grass-legume pastures, applying

dolomitic limestone, and controlling K and N fertilization practices.

Hypokalemia

Recent reports have recognized severe postpartum hypokalemia (1.4 to 2.3 mEq/L; normal range: 3.9 to 5.8 mEq/L) in dairy cattle as another possible metabolic cause of muscle weakness and alert downer cows.[120–122] Moderate hypokalemia (serum K concentration of 2.4–3.8 mEq/L) often is secondary to a disease process that reduces feed intake or intestinal and renal disease causing increased body K loss. Rarely is disease-induced hypokalemia significant enough to result in profound muscle weakness and recumbency. Common clinical signs included profound flaccid muscle weakness or recumbency, variable degrees of anorexia, and cardiac arrhythmias. The most common clinical chemistry findings include alkalosis and elevated muscle enzymes CK and AST. In one study, other possible causes of recumbency were not found, with the exception of severe hypophosphatemia in four cases.[120] In 92% (23 of 25) of cases cited in two reports,[120,122] a previous history of ketosis and repeated ketosis treatments were reported. In a third study, protracted disease, often infectious in origin, was reported as the most common historical event.[121] No age predilection across cases was found, but most commonly the hypokalemic event occurred in early lactation, typically before 60 days in milk. Multiple corticosteroid therapies or dosing beyond recommended amounts was a common historical finding. Isoflupredone acetate was administered in 79.5% (31 of 39) and dexamethasone in another 7.7% (3 of 39), and no corticosteroid was used in 12.8% (5 of 39) of reported cases in these studies.[120–122]

K is the predominant intracellular cation and serves many biologic functions including acid-base balance, osmotic pressure regulation, and water balance.[123] Only 2% of total body K is located extracellularly, with the remainder located in muscle. This differential distribution across cell membranes is responsible for resting electrical membrane potentials, and changes in this electrical potential are responsible for nerve cell conduction and muscle contraction.[123]

Intestinal absorption efficiency of dietary K exceeds 90%.[124] Forage-based diets then greatly exceed daily K requirements, thus necessitating a mechanism for excretion to prevent cardiotoxic hyperkalemia. Homeostatic regulation is weakly managed by aldosterone's effects on stimulating K excretion from colon and kidneys.[111,123] Potassium regulation becomes more convoluted, because muscle and liver cells can rapidly remove extracellular K into intracellular compartments. Insulin and β-adrenergic agents can facilitate intracellular movement, and acid-base status further modifies these relationships. K^+ moves either into or out of cells in exchange for H^+, in an effort to maintain proper blood pH.

In view of these regulatory relationships in which the system is essentially balanced to manage excessive K intake, early-lactation cows, which lose 1.5 g/kg K into milk[124] and have marginal DM intake at best, potentially could be at risk for hypokalemia under the right conditions. Factors increasing extracellular K removal, by either excretion or intracellular movement, exacerbate risk for hypokalemia. Corticosteroids with mineralocorticoid activity, such as isoflupredone, potentiate renal excretion of K. This was a common observation in reported studies.[120–122] A majority of hyperkalemic cows also were alkalotic, developed ketosis, and were treated multiple times with glucogenic compounds and insulin. All of these factors will cumulatively contribute to greater K loss and account for observed severe hypokalemia.

Diagnosis of severe hypokalemia is made on the basis of presenting signs, measured blood K concentration below 2.3 mEq/L, and absence of other factors responsible for recumbency. Characteristic myodegenerative changes with vacuolation can be seen in muscle biopsy specimens.[120,123] Treatment should focus on replacing lost K and ensuring a continuing adequate supply. Both intravenous and oral K supplements have been advocated, but reported response to therapy was poor (8 of 25 recovered) in two studies.[120,122] Reported recovery rate was better (11 of 14) in another study.[121] All studies used intravenous and oral K supplementation, but with different regimens. Intravenous K administration should not exceed 0.5 mEq/kg/hour to avoid potential cardiac toxicities.[111] Oral K supplementation can be administered as 100 to 150 g potassium chloride twice daily.[111] Mineral supplement should be mixed into several gallons of water to be administered. Including oral glucose precursors (calcium propionate or propylene glycol) or concurrent intravenous dextrose therapy can augment cellular K uptake by stimulating insulin release. Prevention of this syndrome evidently is more management related than nutritional, so long as cows have adequate feed intake. Flagrant use of corticosteroids should be discouraged. Aggressive oral K supplementation for anorectic early-lactation cows with typical postparturient problems may have some value, and further evaluation is warranted.[111]

DISEASE ASSOCIATED WITH IMMUNE DYSFUNCTION

Immune function of the dairy cow has been shown to decline in responsiveness during the transition period.[125–127] Kehrli and colleagues showed that both neutrophils[128] and lymphocytes[129] collected from periparturient cows had reduced immunologic responses immediately after parturition. More recent work has identified down-regulation of glucocorticoid receptors on neutrophils and mononuclear cells around the time of calving, which was proposed to be responsible for altered cell function and associated immunosuppression.[130,131] Stress factors that increase corticoid secretion can further down-regulate immune cell receptors and consequently impair cell function. In elegant studies in which mastectomy was performed on late-pregnancy cows, alterations in lymphocyte cell populations, impaired lymphocyte function, and reduced neutrophil bactericidal activity all were ameliorated in mastectomized compared with intact cows.[132–134] These findings suggest that metabolic or physiologic losses associated with initiation of lactation may account for part of the immunosuppression experienced

by periparturient cows. Although hormonal and metabolic factors may possibly play a primary role in this physiologic immunosuppression, it can be further suppressed by dietary insults. Energy, protein, macro- and microminerals, and fat-soluble vitamins all are potential nutritional mediators of immune function.[135]

A compromised immune system is associated with increased risk of metritis,[136] mastitis,[134,138] or other infectious disease process. Retained placenta recently has been characterized as a failure of the maternal immune system to adequately recognize fetal antigens to initiate the separation of maternal and fetal placental components.[134] Cows experiencing negative energy or protein balance are more predisposed to the development of infectious diseases. Relationship between vitamin E and selenium status and mastitis[139–141] and retained placenta[142,143] incidence has been well documented. Vitamin E and selenium supplementation was found to improve bactericidal activity of neutrophils, which may account for their effect on reducing mastitis incidence and severity.[138] Prepartal vitamin A status also has been shown to be protective for mastitis.[143] Parenteral vitamin E supplementation has been shown to reduce metritis incidence.[142] Activated vitamin D (1,25-DHCC) was shown to augment antibody production response to *Escherichia coli* vaccination against mastitis.[144] Efforts to minimize infectious problems during the transition period need to address factors associated with reducing the infectious agent challenge in the environment, improving immune responses (see "Maintaining Immune Function" section later on) and reducing stress.

DISEASE ASSOCIATED WITH RUMEN DYSFUNCTION

Etiologies of previously reviewed periparturient diseases were associated with deranged homeostasis of essential nutrients required in support of maternal metabolism. The rumen fermentation system is first to process consumed feeds and, through its microbial activity, provides a substantial portion of required nutritive substrates to support the host cow's needs. Ruminal microbial populations require a variety of nutrients in support of their metabolism and growth. Similar to the host cow, microorganisms require substrates to provide energy, nitrogen for protein synthesis, minerals, and vitamins. Tremendous variation exists among bacterial species in desired forms of nutritive substrates. Unique to the rumen system is a requirement for fiber in the form of complex carbohydrates comprising the plant cell wall that are indigestible to the cow. Dietary fiber content and form, in conjunction with feeding management practices, can determine success or failure of rumen function during the transition period.

Left Displaced Abomasum

Physical displacement and entrapment of the abomasum to the left side of the rumen (i.e., LDA) constitute a common disease of dairy cattle. Nearly 90% of LDA occurrences are diagnosed within 1 month of calving, with a majority occurring within the first 2 weeks.[145] Abomasal entrapment is thought to be caused by a combination of reduced rumen size as a result of lower intake, physical displacement of the rumen by the gravid uterus, and abomasal atony and gas accumulation.[146] Because this disease occurs soon after calving, transition nutrition is key to causation and prevention. Identified nutritional risk factors include amount of grain fed in the prepartal or postpartum diet, dietary fiber content and form, dry cow forage program, and feed bunk management (e.g., feed availability, abrupt feed changes, feed sorting potential).[145] Cows with high BCS at calving were predisposed to development of LDA,[147] as well as cows with twin pregnancy.[148,149] One or any combination of these factors could account for lower feed intake and subsequent reduced rumen fill, thus contributing to LDA prevalence.

Elevated blood NEFA concentration, a measure of negative energy balance and a reflection of low feed intake, has been associated with risk for LDA.[147,149] Similarly, a number of periparturient diseases have been associated with increased LDA risk. Cows with ketosis, metritis, and retained placenta all were at higher risk for LDA.[57,148–150] Again, the association between these diseases may be mitigated by adverse effects on DM intake. Ketosis is highly associated with LDA occurrence, evidenced by the eightfold higher risk for LDA in cows with serum BHB concentration 12.5 mg/dl or greater.[149] A similar risk between ketosis and LDA was found using milk ketone concentration.[92] Parturient paresis[148,150,151] and subclinical hypocalcemia[98] were found to increase LDA risk. Intuitively, hypocalcemia and its potential adverse effect on abomasal motility seem plausible. No association between blood Ca concentration and LDA incidence was found, however, when negative energy balance measures (NEFA, BHB) were evaluated. This observation suggests that the effect of hypocalcemia on LDA incidence was secondary, a result of reduced intake, rather than directly causal.[149,152]

Cows fed a diet to induce fatty liver infiltration (high energy, low protein) experienced a 25% LDA prevalence and had severe fatty liver with accompanying indicators of liver dysfunction and negative energy balance.[153] Cows with LDA from this study had a 56.6% reduction in DM intake during the 10 days before LDA diagnosis, compared with cows without an LDA. Although a causal relationship between negative energy balance and LDA incidence cannot be established, practices aimed at preventing negative energy balance and ketosis should provide some protection against LDA incidence.

Subacute Ruminal Acidosis

Ingestion of large amounts of readily fermented carbohydrates results in excessive production of VFAs and lactic acid beyond the capacity of epithelial cell absorption, lactate-utilizing bacteria, and rumen buffers to ameliorate a dramatic decline in the pH of ruminal contents. Clinical forms of ruminal acidosis can manifest as peracute, acute, or subacute disease processes. All forms may occur in any ruminant animal, but subacute ruminal acidosis (SARA) is most common and an economically

important disease of feedlot[154] and dairy[155,156] cattle. Although data are limited, prevalence of SARA has been reported to be as high as 40% of animals in specific herds, and across 15 herds, 19% and 26% of early- and mid-lactation cows, respectively, were affected in one study.[157]

Peracute and acute forms of ruminal acidosis (e.g., grain overload, carbohydrate engorgement) result in a life-threatening syndrome characterized by prolonged and dramatic decline in rumen pH (<4.5), systemic dehydration, and uncompensated metabolic acidosis from absorbed D-lactate.[158] Although ruminal acidosis is an important disease process, the peracute and acute forms of acidosis are sporadic individual or herd problems resulting from errors in feeding management or accidental exposure to high-grain-content diets. More detailed discussion of acute acidosis can be found elsewhere.[154,158,159] Of greater practical concern in both feedlot and dairy operations is the prevalence of SARA. Similar in pathogenesis to acute ruminal acidosis and situated along a continuum of problems involving duration and severity of ruminal pH reductions, SARA is defined as transient declines in rumen pH to between 5.0 and 5.5 observed after feeding bouts.[155,156,160] A similar definition could be attributed to chronic ruminal acidosis; however, the discriminatory issue between subacute and chronic acidosis is duration (hours versus days, respectively) of low-pH events.

Collective populations of microorganisms within the rumen environment are capable of using a tremendous variety of dietary substrates to support their metabolic functions and growth and to provide usable end products for support of host animal metabolism. Individual microbial species, however, have a more narrow substrate requirement, assigning them to a somewhat exclusive niche within the collective rumen environment. Interdependence of species occurs wherein one microbial group uses fermentation end products from another group or generates necessary metabolic cofactors for a different group. Most rumen microbes are pH sensitive, requiring an environmental pH of 6.0 or higher, especially for cellulolytic bacteria. The goal of feeding ruminant animals is to maximize microbial fermentation of fibrous feed sources in support of high-quality meat and milk production. Because fibrous feeds will not support high levels of productivity, use of concentrated energy feeds is necessary. Sugar- and starch- (i.e., NSC) fermenting bacteria have rapid rates of growth and generate lactate, which disproportionally contributes to low rumen pH. Diets containing large amounts of readily fermentable feed substrates will enhance growth of these NSC bacteria, with the potential for altered rumen pH and adverse effects on fiber fermentation.

With any dramatic change in dietary feed ingredients or feed intake, there will be a period of adaptation by microbial populations until an optimal steady state is achieved. The objective of any feeding program should be to minimize deviations in ruminal pH. Unfortunately, the transition cow experiences numerous changes in dietary composition, reduced feed intake, and rapid exposure to large amounts of highly fermentable carbohydrates. The transition cow is inevitably at high risk for the development of SARA. Although microbial adaptation and rumen epithelial growth relative to sensitivity to SARA are pervasive, recent studies have not shown a significant effect of prepartal grain feeding on prevention of SARA.[157,161] This evidence suggests that the amount of consumed fermentable carbohydrate, with its inherent ability to ferment rapidly and overwhelm rumen buffer systems, is the primary mediator of SARA.

Clinical signs associated with SARA often are subtle and may not be seen until later in the clinical course. Affected cows also may show signs of laminitis-induced lameness,[162] reduced milk fat content and milk production, low and cyclic ("slug" feeding behavior) feed intake, loss of body condition, and loose or variable fecal consistency.[155,156,160] Many of these clinical signs can be attributed to other disease problems, but chronic laminitis within a herd should place SARA high on the differential diagnosis list.[155,162] As low ruminal pH episodes continue over time, pathologic changes consistent with chronic acid exposure can occur. Rumen papillae may undergo hyperkeratosis with matting, ultimately leading to ruminal wall ulcer formation with secondary mycotic or bacterial colonization.[158] Bacterial translocation across ruminal wall lesions into the portal vein will result in hepatic abscesses. More severe repercussions such as caudal vena cava syndrome, hemoptysis, and epistaxis can occur in some herds secondary to ruminitis–hepatic abscess complex.[155]

Diagnosis of SARA is accomplished by measuring rumen pH after feeding and finding pH values below 5.5. Oetzel[29] suggests obtaining samples from 12 animals within a group; in this approach, if three or more cows have pH values below 5.5, the group is considered to be at high risk. Analysis of samples obtained by means of percutaneous rumenocentesis has been advocated as the preferred method for accurate determination of pH.[155,163] Samples collected using orogastric tubes or specialized ruminal fluid collection devices generally have various degrees of saliva contamination elevating rumen fluid pH values by 0.5 to 1.0 unit.[164]

Dietary evaluation should be used to complement measurement of ruminal pH, to determine any potential problem source. Chemical analysis of feed ingredients and delivered diet should be undertaken to determine total content of fermentable and fibrous carbohydrates. Estimation of effective fiber and overall dietary particle size also can be done.[165] Grain sources can be sieved to determine particle size distribution, as a method to evaluate ruminal degradation ability. More important, dietary particle separation should be used to evaluate delivered dietary consistency and cow sorting behavior to better evaluate the consumed diet.

Prevention of SARA essentially revolves around proper management of dietary carbohydrate delivery and consumption. Carbohydrates that potentially contribute to lactic acid production (i.e., sugars, starches, fructosans) need to be controlled in their dietary content and inherent rate of degradation.[156,160] Starch source, hydration, particle size, and processing all can influence rate of microbial degradation, leading to more rapid production of VFA and lactic acid. Combining starch hydration (ensiling) and particle size reduction (grinding) can

greatly increase rate of ruminal degradation and risk of acidosis. An adequate amount of physically effective fiber must be consumed from the diet to ensure good rumination and saliva production. Ration particle separation should be used to evaluate proper distribution of particles and to determine whether sufficient amount of effective fiber is being consumed. Fermentable fiber sources (e.g., beet or citrus pulp, soybean hulls, wheat middlings) often can be used to replace a portion of the dietary NSC fraction, thereby reducing lactic acid load yet providing energy in support of microbial growth and milk production.

Feeding management practices are just as important as dietary composition, if not more so. Ensuring consistent feed availability and composition, adequate bunk space and minimization of environmental stressors will help to control variability and dramatic feed intake changes. These issues are especially challenging over the transition period. Use of ruminal buffers (sodium bicarbonate, magnesium oxide) in early-lactation rations is highly recommended.[166] Addition of yeast-based probiotic products has been shown to help maintain rumen microbial population stability and feed intake.[167] Recently the ionophore agent monensin sodium (Rumensin, Elanco) has been approved for use in lactating dairy cattle rations. Ionophores selectively inhibit gram-positive bacteria, especially those associated with fiber and starch fermentation, resulting in reduced lactate production and protection against ruminal acidosis.[168]

KEY CONTROL POINTS FOR TRANSITION SUCCESS

In preventing associated problems with periparturient metabolic disease, the focus must be on controlling but not eliminating negative energy balance, minimizing fatty infiltration of the liver, and maintaining normal Ca homeostasis. Reducing the incidence of hypocalcemia, ketosis, and fatty liver syndrome will go a long way toward preventing or minimizing other parturient problems such as mastitis, metritis, retained placenta, and LDA. To prevent periparturient disease, four critical control points during the transition period need to be addressed:

Optimizing DM intake
Feeding a balanced diet
Maintaining Ca homeostasis
Minimizing immune system dysfunction[126]

Optimizing Dry Matter Intake

Cows that experienced periparturient disease have been shown to have a greater decline in prepartal DM intake compared with unaffected cows.[169,170] Cows experiencing postpartum disease also continued to have lower postpartum DM intake compared with healthy cows.[171] It also is well documented that DM intake normally declines by 25% to 30% in late gestation.[124,172,173] Timing relative to parturition and severity of the DM intake decline in late gestation varies among individual animals within a group and between groups within herds. An interplay of various physiologic (e.g., physical distention) and metabolic (e.g., hormone and metabolite concentrations) factors primarily regulates intake capacity. Other factors, however, including diet composition (e.g., effective fiber, fiber quality), pregnancy status (twins versus singletons), parity, BCS, presence of inflammatory or immune mediators, environmental conditions (e.g., heat stress) and management (e.g., overcrowding, pen changes, feed availability) may further influence actual DM intake in late pregnancy and early lactation.[173–175] Cows with higher body condition (mean BCS of 4.4 of a maximum 5.0) had lower mean DM intake (1.68 versus 1.83% of body weight) across the prepartal period and a more dramatic decline (40% versus 29%) compared with moderate (BCS of 3.6/5.0) or thin (BCS of 2.8/5.0) body condition cows.[173] Mature Holstein cows pregnant with twins (13.1 kg/day, 1.8% of body weight) had lower DM intake compared with singleton-pregnant cows (14.9 kg/day, 2.0% of body weight) across the prepartal period.[176] These data may help explain why heavy condition and twin pregnant cows have greater incidence of periparturient diseases and suggest the need for a special dietary group in the prepartal period to account for altered intake. Similarly, first-lactation cows have lower intake capacity and would benefit from a diet tailored to their intake, rather than diets formulated to meet mature cow intake capacity.

Lower prepartal DM intake will result in a greater degree of negative energy balance placing cows at greater risk for excessive hepatic fatty infiltration and impending downward spiral into other associated periparturient diseases. In a classic study by Bertics and colleagues,[172] rumen-cannulated cows that had their refused feed placed through the rumen cannula had lower blood NEFA concentrations and less fatty infiltration of the liver compared with cows allowed to refuse feed, resulting in reduced total intake. Application of these data indicated that maximal prepartal DM intake would be encouraged by feeding amounts to ensure 5% to 10% refusals for a group. Alternatively, dietary energy and protein content can be increased to compensate for lower DM intake.[47]

Besides a potential role in preventing periparturient disease, maximizing prepartal intake is thought to help accelerate DM intake post partum. Prepartal and postpartum intake data from multiple studies showed a positive relationship (r = 0.54) between intake during the last week of pregnancy and subsequent early postpartum DM intake.[177] Efforts to increase postpartum DM intake will facilitate energy balance status, minimize risk for postpartum disease, and support milk production. Properly balanced diets and removal of obstacles impeding DM intake will promote consistent high intake, which is most desirable.

Although arguments for maximizing DM intake and cow health are persuasive, there may be practical limitations and consequences. Other studies[178–181] and a review of data[182] suggest that moderate prepartal feed restriction may increase DM intake post partum. Cows moderately restricted in feed intake (80% of energy requirement) had blunted prepartal NEFA curves, less hepatic fat content, and greater postpartum intake. Grummer and associ-

ates[182] suggest that this situation mimics that of first-lactation cows, which seem to be more resistant to hepatic lipid accumulation despite negative energy balance. Observations suggest that feed-restricted cows and heifers do not experience the dramatic decline in intake accounting for this response. Drackley proposed that the extent of prepartal DM intake reduction was more important than the amount of prepartal intake on postpartum DM intake and hepatic fat accumulation.[183] The message from these studies may be not so much that intake must be maximized, but rather that the potential for significant declines in feed intake around transition should be minimized. The gold standard in feeding the transition cow, however, would be to achieve consistently high prepartal and postpartum intakes, accomplished through well-balanced diets and good management practices.

Feeding Properly Balanced Diets

A successful transition cow program can be developed using any of a variety of methods. Diets must be appropriately formulated to work within the framework of the physical environment, management practices, observed intake, and available feed ingredients. Cow grouping strategies and intake potential probably are the most critical to success of the formulation process. The more cow groups can be separated by intake and nutrient needs, the more precise the nutritional program becomes in meeting all animals' nutrient needs. As groups are reduced across parity and days before parturition, greater variation in nutrient intake on an individual animal basis is introduced, increasing opportunities for metabolic disease problems. The current NRC dairy cattle publication has greatly improved its recommendations relative to transition dairy cows and should be used as a guideline[124] (Table 46-3).

Of primary concern is providing the appropriate balance between dietary fibrous and readily fermentable carbohydrates. Dietary fiber sources provide substrate to support cellulolytic activity, and the physical properties of fibrous feeds promote rumination, facilitate rumen buffering, and provide material for the fibrous rumen mat. As a result of their slower rate of fermentation and requirement for repeated mastication to reduce particle size, fibrous feeds limit intake capacity and dilute dietary energy density. Cereal grains and other readily fermentable carbohydrate (NSC) sources provide readily available energy to support rumen microbial growth and the cow. Theoretically, feeding of some NSC sources helps

Table **46-3**

General Nutrient Recommendations (Dry Matter Basis) for the Transition Cow

Nutrient	Parity	DRY COWS		Fresh Cow (1–21 d)
		Early	*Close-up*	
Dry matter intake, % of body weight	Heifers	1.7–1.9	1.4–1.6	>2.2
	Mature cows	1.9–2.1	1.6–1.8	>2.5
Net energy, Mcal/lb	Heifers	0.60–0.64	0.70–0.72	0.78–0.80
	Mature cows	0.55–0.60	0.68–0.71	0.77–0.79
Acid detergent fiber, %		21–30	21–25	17–21
Neutral detergent fiber, %		38–45	33–38	25–33
Neutral detergent fiber, % of body weight	Heifers	0.8–1.0	0.6–0.7	0.85–0.95
	Mature cows	0.9–1.1	0.7–0.9	0.95–1.05
Nonfiber carbohydrates, %	Heifers	29–32	35–40	36–38
	Mature cows	28–30	34–38	36–38
Crude protein, %	Heifers	13–14	15–16	18–19
	Mature cows	12–13	13–14	17–18
Soluble crude protein, %		30–35	28–32	30
Rumen-degradable protein, %		68–72	64–67	60
Rumen-undegradable protein, %		28–32	33–36	40
Metabolizable protein, g/d	Heifers	900–1000	1000–1100	1200–1800
	Mature cows	850–950	1050–1150	1700–2300
Calcium, %		0.4–0.6	0.6 (1–1.2)[†]	0.85–1.0
Phosphorus, %		0.25–0.30	0.30–0.40	0.35–0.40
Magnesium, %		0.2–0.25	0.35–0.40	0.25–0.3
Potassium, %		0.65–1.0	<1.5	1.2
Sodium, %		0.10	0.1	0.3–0.35
Chloride, %		0.15	0.15 (0.8)[2]	0.30–0.35
Sulfur, %		0.16–0.2	0.2 (0.4)[2]	0.2–0.25

*Adjusted for anionic salt diets to achieve desired pH effect.

Data from National Research Council: *Nutrient requirements of dairy cattle,* 7th rev ed. Washington, DC: National Academy Press, 2001; Van Saun RJ: Dry cow nutrition: The key to improving fresh cow performance. *Vet Clin North Am Food Anim Pract* 1991;7:599–620; and Hutjens MF: *Hoard's dairyman feeding guide.* Ft. Atkinson, WI: WD Hoard's & Sons Comp, 1998, p 73.

the rumen papillae, as well as the rumen microbial population, to adapt to higher-grain-content diets. Excessive NSC, however, can result in acidosis and excessive body condition gain. Use of fat as an energy source should be limited in transition cows without exceptional feeding management, because of adverse effects of fat on DM intake and rumen fermentation or a lack of beneficial effect on health and production.[8,184,185] Fermentable fiber sources are receiving much attention in transition diets, because they can replace NSC ingredients to provide readily fermentable energy but do not have a negative effect on DM intake, as do other fibrous sources.[186] A minimum amount of physically effective fiber must be supplied in the diet, however, to maintain good rumen function and fill.

In balancing feed ingredients to meet dietary fiber and energy needs, feed nitrogen sources need to be balanced to meet rumen microbial nitrogen needs (rumen-degradable protein should be 11% of DM) to support microbial protein synthesis. The cow's protein requirement is best gauged by metabolizable protein, which is the sum of digestible amino acids delivered to the small intestine from microbial and feed protein sources. Too often (especially with hay crop silage diets), dietary crude protein content is much higher than needed (12–14%), yet predicted metabolizable protein is less than estimated requirements.[187] Unfortunately, metabolizable protein cannot be measured directly in feed ingredients. Metabolizable protein delivery from a given diet must be predicted using sophisticated dynamic rumen models such as the NRC dairy[124] or beef[188] cattle software package or Cornell-Penn-Miner program. Hay crop forage-based diets often are unbalanced relative to rumen-available nitrogen and readily available energy, resulting in predicted low metabolizable protein amounts. Adding fermentable carbohydrates or corn silage can potentially better balance the diet for metabolizable protein.

Maintaining Calcium Homeostasis

Most factors responsible for predisposing the cow to parturient hypocalcemia (age, breed, milk production) are not easily altered to reduce incidence, with the exception of prepartal diet. Beyond initial attempts at manipulating dietary Ca-to-P ratio, reducing dietary Ca and P intakes, and various modes of supplementing vitamin D metabolites or their analogues, manipulating prepartal DCAD shows the greatest opportunity for a practical method of hypocalcemia prevention.[95,111,189,190] Boda and Cole[191] first proposed manipulation of dietary Ca-to-P ratio to control milk fever incidence. To achieve the desired 1.5:1 to 2.0:1 ratio, dietary P often was fed in excess of requirements. Kirchura and co-workers[192] showed that high P intake increased the risk of parturient hypocalcemia. Massive doses of vitamin D (>1 million units/day) were advocated,[193] but problems with timing of administration relative to calving, recurrent hypocalcemia post partum and toxicity concerns were problematic.[194] Research studies showed potential application of prolonged-release vitamin D analogues[195] or PTH.[196] Neither protocol has been translated into a practical method to be applied in clinical practice.

Targeted Calcium Therapy

Although bordering on therapy (see previous section on hypocalcemia) rather than prevention, supplementation of soluble Ca sources around the time of calving can help to minimize parturient hypocalcemia. Administration of subcutaneous Ca solutions to all mature cows at the time of calving has been used to minimize potential for subclinical hypocalcemia. Alternatively, oral Ca salts can be given. It is not advisable to administer both Ca sources, because the potential for toxicity is increased. Highly soluble Ca salts are necessary to provide sufficient ruminal Ca concentration to passively drive Ca between epithelial cells. Calcium chloride (36% Ca), propionate (21.5% Ca), gluconate (9.3% Ca), and borogluconate (8.3% Ca) formulations can be used as oral supplements. Dosage should be between 50 and 125 g Ca.[111] Toxicity may occur with Ca dosages in excess of 250 g.[40] To prevent parturient hypocalcemia, mature cows can be dosed by commercial oral gels, drench, or stomach tube with an appropriate soluble Ca source just before or at the time of calving. Additional dosing could be administered once or twice after calving at 24-hour intervals. If cows become recumbent or fail to respond adequately, further diagnostic evaluation should be undertaken.

Dietary Calcium Content

Following the inability to prevent parturient hypocalcemia by manipulating dietary Ca-to-P ratio, preventive emphasis should focus on absolute dietary Ca intake. Assuming that the Ca homeostatic response was blunted or delayed when cows were fed Ca in excess of prepartal requirement, efforts were directed at reducing prepartal dietary Ca. Two studies feeding very low dietary calcium (<20 g/day) for 2 weeks before calving showed complete prevention of mild fever.[197,198] These diets provided less than required absorbed Ca (22 g/day in Holsteins)[124] and thus induced activation of the Ca homeostatic system to maintain blood Ca concentration. Unfortunately, routine formulation of such diets with available feedstuffs on most dairy farms is not practical. The successful reduction in milk fever observed in these studies, however, led to general recommendations of feeding reduced Ca (<80 g/day).[199] Field results for this preventive approach were variable and often did not reduce subclinical hypocalcemia. Later studies failed to show any effect of prepartal dietary Ca intake on milk fever incidence.[200–202] Other confounding factors such as effects of dietary K and Mg most likely accounted for differing responses. Successful use of low-Ca diets is accomplished by concurrently supplementing dietary Mg intake (0.35% to 0.4% DM) and reducing dietary K (<1.5% DM). Mineral sources containing Ca should be removed from the diet, so that most of the dietary Ca is derived from forage sources, which will provide less absorbable Ca intake.

As previously stated, formulating prepartal diets for extremely low Ca intake with typical feeds is difficult at best. An alternative to reducing dietary Ca content is to reduce dietary Ca availability with binding agents such as vegetable oil and sodium aluminum silicate (Zeolite A). Vegetable oils can generate insoluble Ca soaps, as well as Mg soaps, and may adversely affect prepartal DM intake and energy metabolism. Zeolite added to prepartal diets

has been shown to reduce parturient hypocalcemia,[203,204] but it is a nonspecific binding agent and must be added in significant amounts (0.7–1 kg/day). Both blood P and Mg concentrations were reduced in cows receiving diets supplemented with zeolite showing the nonspecific binding properties.[204] Binding agents are best used when dietary Ca content is already marginally low (30–50 g/day) to help attain the desired amount of absorbable Ca intake (<25 g/day). Zeolite may be of benefit in controlling hypocalcemia in pasture-based feeding systems to reduce absorbable Ca intake, because reducing K intake on pasture is less manageable.[205]

Dietary Cation-Anion Difference

The role of systemic acid-base status mediated by the relative difference between strong dietary cations (Na, K) and anions (Cl, S) in Ca homeostasis and as a mechanism for milk fever prevention has been well studied (see available reviews[95,190,206,207]). It is proposed that marginal differences in systemic pH influence the interaction between PTH and its target organ (bone, kidney) receptor. Metabolic alkalosis induced by an excess of absorbable cations (positive DCAD) diminishes PTH's stimulation of renal vitamin D activation, resulting in perturbed Ca homeostasis.[95,111] Inducing a state of metabolic acidosis by feeding an excess of absorbable anions (negative DCAD) augments Ca mobilization from labile bone. Additionally, activation of vitamin D is enhanced resulting in greater efficiency of intestinal Ca absorption.

The ubiquitous equation used to describe DCAD is (Na + K) − (Cl + S), with all values given in milliequivalents (mEq) per 100 g or kg of dietary DM. Factors used to convert dietary mineral content to mEq units are shown in Table 46-4. A number of alternative equations to describe DCAD have been proposed, accounting for the contribution of all potential dietary cations and anions and their availability.[95,207] Recommendations for desired DCAD to evoke an appropriate response, however, are equation specific, and definitive criteria for these alternative equations have not been established. Using the simple equation accounting for Na, K, Cl, and S, a desired range for DCAD to prevent milk fever is between –50 and –100 mEq/kg DM.[95] This is slightly less negative than what has been recommended (–150 mEq/kg DM),[206] and the current trend in the field is not to reduce DCAD to such low levels, owing to negative effects on DM intake and energy balance. Summarizing multiple studies, Oetzel showed an 11.7% reduction in DM intake when DCAD was reduced by 300 mEq/kg.[206] Palatability of anionic mineral salts has been a concern[208,209]; however, hydrochloric acid–treated protein products seemingly have alleviated some of this problem.

Application of the DCAD concept initially was daunting as a result of the required mathematical conversions and dietary mineral content bookkeeping for successful milk fever prevention. The primary goal of formulating DCAD-based diets is to remove dietary cations by selecting feed ingredients with lower K content and minimizing Na and K mineral or additive sources. This is followed by addition of dietary anion sources (mineral salts or acid-treated supplements) to achieve the desired cation-anion difference. Chloride-based salts seem to have the greatest impact on altering acid-base status.[95,111] Response to cation-anion manipulation of the diet can be easily monitored by measuring urinary pH.[210] Urinary pH is sensitive to acid load and is highly correlated ($R^2 = 0.77$) with change in blood pH.[206] Desired range in urinary pH to achieve sufficient metabolic acidosis to prevent milk fever is 6.5 to 7.0 in Holsteins or 6.0 to 6.5 in Jersey cattle.[111,206] Urinary pH values below 5.5 suggest excessive acidification, and some anions should be removed from the diet.

Although all macrominerals potentially mediate Ca homeostasis, practical experience with urinary pH measurements and rationalization of mineral requirements relative to their contribution to acid-base status has simplified the application of DCAD to milk fever prevention.[111] Dietary Mg should be increased to 0.35% to 0.4% DM to counter antagonism from higher dietary K content and to ensure adequate Mg to support the Ca homeostatic system. Because of inhibitory effects of high blood P concentration on vitamin D activation and inherently

Table **46-4**

Equivalent Values for Macrominerals and Content-Weight Conversion Factors for Calculating Cation-Anion Difference*

					CONVERSION TO:	
Mineral	**Atomic Wt (g/mol)**	**Valence**	**Eq Wt (g)**	**mEq Wt (g)**	**mEq/100 g**	**mEq/kg DM**
Sodium (Na)	23.0	+1	23.0	0.023	43.48	434.78
Potassium (K)	39.0	+1	39.1	0.039	25.64	256.41
Chloride (Cl)	35.5	−1	35.5	0.0355	28.17	281.69
Sulfur (S)	32.0	−2	32.1	0.016	62.50	625.00
Calcium (Ca)	40.1	+2	40.1	0.02005	49.88	498.75
Magnesium (Mg)	24.3	+2	24.3	0.01216	82.24	822.37
Phosphorus (P)	31	−3	31.0	0.01033	96.81	968.05

*Factors used to convert dietary mineral content (% DM) to equivalent weights.
DM, dry matter.

low acidifying effects, there is no need to increase dietary P beyond current NRC[124] recommendations (0.3–0.35% DM). Dietary sulfur (S) should be supplemented between 0.22% and 0.4% DM. In view of the questionable acidifying effect of sulfate ions and the potential toxicity of S (e.g., polioencephalomalacia),[211] supplementing to greater than this level is not recommended. Na requirement can be met with a diet containing 0.12% Na.[124] Forages typically are low in Na (<0.1%), and minimal salt could be added to the diet to meet requirements. Suggested Ca content of anionic diets was thought to be in excess of requirement to account for induced renal Ca diuresis with acidosis. Calcium intake in excess of 150 g/day often was recommended; however, Beede and colleagues showed reduced DM intake with anionic diets containing 1.5% or 1.95% Ca, and blood Ca concentration was reduced on diets containing 0.47% Ca.[212] Recommended dietary Ca content with anionic diets is between 0.85% and 1.2%.[111,212] Exclusive use of calcium carbonate as the Ca source is to be avoided, owing to its potential alkalizing effects. This approach then reduces dietary manipulations to achieve a desirable DCAD to variability in dietary K and Cl content. Monitoring forage K content, application of agronomic practices to reduce forage K content, and substituting low-K by-product feeds for forages are potential methods to reduce dietary K content. Chloride sources from mineral salts or acids can be titrated into the diet to achieve the desired effect as measured by urinary pH change. As a general guideline, dietary Cl content should be 0.5 percentage unit less than dietary K content.[111] Acidogenic diets should be fed for at least 10 days, and preferably 2 to 3 weeks, before calving, to achieve the best preventive response.

Maintaining Immune Function

Based on the information presented, a decline in immune cell functional capacity to control infectious agents is inevitable with initiation of lactation. Stressor increasing cortisol production and inadequate nutrition can further exacerbate the duration and severity of immune suppression. Cow management during the transition period should focus on minimizing pen moves, because such changes force reestablishment of social order among individual animals, resulting in increased cow-to-cow interactions and lower DM intake.[62] Parturient cows can be moved into maternity pens only to deliver the calf and then moved to a postfresh pen, in an effort to decrease pen moves. Overcrowding of prefresh, maternity, and immediate postfresh groups can alter behavior (i.e., stress) and decrease DM intake. Based on differences in girth for pregnant cows, greater bunk space per cow is suggested (27 to 30 inches per cow), and a stocking density of 85% to 90% is recommended.[62] Environmental management to address pathogen exposure and ambient temperature extremes also is necessary to reduce potential stressors.

First and foremost, negative energy balance and protein deficiency need to be minimized throughout the gestational period, in accordance with the feeding recommendations described previously. Second, gestational micromineral and vitamin losses to fetus and colostrum may significantly affect maternal reserves and their metabolic function, especially when mineral and vitamin supplementation is reduced or interrupted during the dry period. In view of the proven role of trace minerals and fat-soluble vitamins in modulating immune function, diets should be fortified to a level at least equivalent to current NRC recommendations. In recognition of data suggesting improved mammary health and milk production effects and to compensate for colostral losses, vitamin A (110 IU/kg of body weight, for a total intake of 70,000–77,000 IU/day) and vitamin E (1.6 IU/kg, for a total of 1000 IU/day) recommendations for transition dairy cows were increased.[124] These are supplemental amounts and do not take into account endogenous vitamin sources in forages. In one study, supplementing vitamin E at 2000 to 4000 IU/day for 2 weeks ante partum, followed by 2000 IU/day for 1 week post partum, resulted in much-reduced mastitis incidence and lower somatic cell counts.[213] Another study also reported improved somatic cell counts in cows receiving 2000 IU/day vitamin E 2 weeks ante partum through 1 week post partum, compared with cows receiving 1000 IU/day for this period.[214] Parenteral vitamin E supplementation (2000–3000 IU total dose), given within 2 weeks of calving, also resulted in improved health status for those cows and heifers with inadequate vitamin E status.[142,215] No data are available to suggest that supplementation of vitamin A above current NRC recommendations has any beneficial effects. Research is only starting to identify animal performance and health responses to supplemental B-vitamins. Anecdotal evidence may suggest that supplementation of probiotic products containing yeast and mixtures of B-vitamins may be beneficial.

Most NRC recommendations for microminerals are based on dietary density, rather than a factorially derived daily amount required, reflecting the paucity of available data. Even though allowances are made for differences in availability depending on dietary ingredients, variation in DM intake from expectations will result in consumption of less mineral. To compensate for intake variation, dietary nutrient density could be increased 20% to 50% above NRC recommendations, or more biologically available mineral sources could be chosen, or some combination of the two approaches could be used. Dietary selenium content cannot be increased above the legal limit of 0.3 mg/kg of diet; accordingly, a portion of the inorganic selenium supplement could be substituted with the yeast-based selenomethionine product to improve availability. Whole-blood selenium and glutathione peroxidase activity was higher at birth in calves from dams supplemented with seleno-yeast than in calves from dams given selenite or no selenium.[216] A suggested starting point would be to replace between 20% and 30% of inorganic mineral sources with available organic forms during the periparturient period. Data in pigs showed improved fetal survival and greater fetal mineral content when a portion (25%) of the trace mineral supplement in the sow's gestational diet was replaced with organic mineral sources.[217] Available data must be evaluated in deciding which organic mineral sources to use and whether or not a response to supplementation is elicited.

Beyond Nutritional Management

In a 1991 survey of 61 top production farms based on DHI records reported mean incidence rates for common periparturient diseases were within expectations; however, the range for each disease was extremely wide.[218] Some farms reported a 44.1% incidence of milk fever or 22.6% incidence of retained placenta. How could farms experience such high periparturient disease rates and still achieve high production? Even with the great strides forward with research on the nutritional management of the transition cow, veterinary science has not solved problems of periparturient disease or reduced reproductive performance. There still is much room for improvement in increasing milk production efficiency. One consistent frustration with managing transition cows is the seeming lack of consistency in response to a given program. To this end, Drackley stated: "Why do vastly different nutrition and management programs produce similarly good, or similarly poor, transition success?"[8] Similarly, Bell, in summarizing the current state of knowledge relative to transition metabolism, suggested that the direction of future research should focus on why individual cows show such wide variation in performance and health responses relative to intake and BCS.[60]

Drackley best answered his own question in suggesting that the traditional approach focusing solely on nutrition is inadequate, and that other important factors include how a given nutrition program is delivered and the environment in which it is consumed.[219] Overcrowding, exposure to pathogens, and changing social organization, among other factors, can induce stress-mediated physiologic and metabolic changes. Immune responses to generate acute-phase proteins and support increased metabolic activity cost energy over and above maintenance requirements. Animals alter how they utilize and partition available nutrients in response to these stress situations, which may compromise availability of nutrients to support productive functions. Other metabolic responses to stress can result in increased fat mobilization, leading to greater potential for hepatic lipidosis, wasting of muscle tissue, and immune suppression. A number of physiologic and metabolic responses to stress can result in a decline in DM intake, further compromising nutrient availability to support production. It is believed that the effects of stressors are additive; thus, as stress situations accumulate, greater physiologic and metabolic changes occur, ultimately resulting in abnormality seen as metabolic dysfunction or infectious disease. These stress responses will be more exaggerated in animals consuming an imbalanced diet but also may overwhelm an animal consuming an adequate diet. The foundation for a successful transition period is the ability to provide a properly formulated close-up ration within a reasonably stress-free environment.

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CHAPTER 47

Ovarian Follicular Cysts

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Ovarian follicular cysts (cysts) are anovulatory follicular structures that persist for various periods of time and have been found in a number of mammalian species.[1] Cysts in most cases are larger than normally developing follicles; however, cysts also may be similar in size to ovulatory follicles. Cysts have traditionally been defined as follicular structures of 2.5 cm in diameter or larger that persist for a variable period in the absence of a corpus luteum (Fig. 47-1). More recently, a diagnosis of ovarian follicular cyst was based on a diameter of 17 mm or larger, particularly if more than one follicular/cyst structure was present.[2,3]

The condition affects approximately 10% of dairy cows annually and is a serious cause of infertility in dairy herds. Occurrence of cysts is less frequent in beef cows, but cysts have become more prevalent in recent years. Cows generally are infertile so long as the condition persists. In our experience, the calving interval is about 50 days longer in cows diagnosed with cysts than in cows without postpartum abnormalities. *Cystic ovaries, ovarian cysts,* and *cystic ovarian degeneration* all are terms that have been used synonymously with ovarian follicular cysts.

This chapter describes ovarian cyst dynamics and reviews the etiology, diagnosis, treatment, and management of cysts in cattle.

OVARIAN FOLLICULAR CYST DYNAMICS

Overview of Follicular Changes during Bovine Estrous Cycle

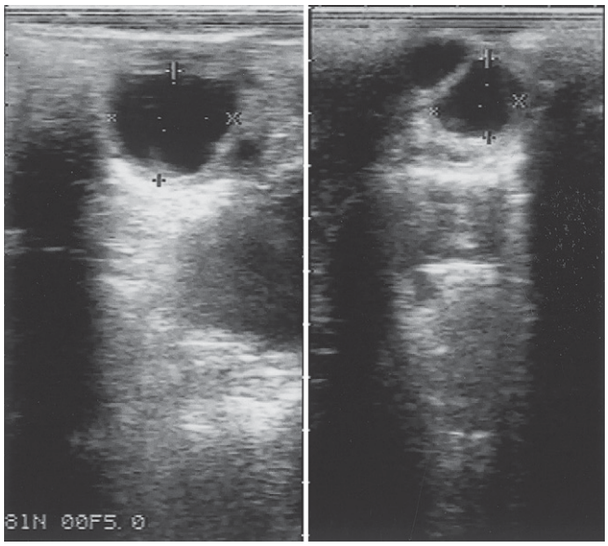
With the development of real-time ultrasonographic technology, ovarian follicular changes during the bovine estrous cycle have been well characterized (follicular dynamics and mechanisms controlling follicular growth and development are reviewed elsewhere[4–9]). The bovine estrous cycle is characterized by growth of follicular waves, usually two or three per cycle. After ovulation subsequent to estrus, a cohort of follicles (usually 3 to 5) is recruited, and these follicles grow to be greater than 5 mm in diameter. Growth continues for approximately 2 days until the follicles reach approximately 8.5 mm in diameter. From the cohort, one follicle is selected to continue growth and become dominant over the other follicles.[10] The fate of the other follicles in the cohort is atresia. If luteal regression occurs during the growing phase of the dominant follicle, that follicle continues growth and ovulates. If luteal regression does not occur during the growth phase of the dominant follicle, the dominant follicle undergoes atresia. Luteal regression usually occurs during the growth phase of the second- or third-wave dominant follicle. Initiation of each wave of follicular growth is preceded by a transient increase in circulating follicle-stimulating hormone (FSH). Initiation of each wave occurs in the absence of a dominant follicle that secretes estradiol and inhibin.

Dynamic Nature of Follicular Growth and Atresia

Ovarian follicular cysts have been described in the literature since the mid 1800s.[11] It was long assumed that most cysts were static structures that persisted for extended periods of time. As indicated by the findings outlined in the previous section, the classic definition of static cystic structures was not consistent with the dynamic nature of follicular growth and atresia. Kesler and associates reported short- and long-term changes in cystic structures and circulating reproductive hormone concentrations that suggested that cysts were not static structures.[12] None of the cows in that study resumed ovulatory ovarian cycles, but cysts appeared to regress and were replaced by new structures in some cows.

Recent studies have shown that cysts are indeed dynamic, with three potential outcomes.[1,10,13–17] In some instances, cysts persist for extended periods (up to 70 days) and remain dominant over the others; growth of additional follicular waves is inhibited by estradiol and inhibin secreted by the active cyst. Two other responses also have been demonstrated[10,13]: The original cyst may regress and be replaced by a new follicular structure that ovulates. Such self-correction occurs in approximately 20% of cows with cysts.[18] The lifespan of most cysts, however, is characterized by turnover whereby the original cyst decreases in size and is replaced by a new follicular structure that develops into a new cyst. With the occurrence of cyst turnover, intervals between follicular/cyst waves are longer and more variable in cows with cysts (13 days) than in cows with normal estrous cycles (8.5 days).

Follicular growth dynamics during development of cysts is similar to recruitment, selection, and dominance of ovulatory follicles during normal estrous cycles.[10] That is, recruitment of a cohort of follicles (usually 3 to 5) is initiated by a transient increase in circulating FSH. The cohort continues to grow until follicles reach 8.5 mm in diameter; then, selection and dominance of one follicle occur. When the dominant follicle reaches ovulatory size, however, ovulation does not occur, and growth of the dominant follicle continues for several additional days



A B

Fig. 47-1 **A,** Ovarian follicular cysts approximately 25 mm in diameter. **B,** Ovarian follicle approximately 17 mm in diameter, which is near ovulatory size

and a cyst develops. The growth rate of follicles that develop into cysts is similar to the growth rate of normal dominant follicles.[10] Cysts that are dominant over other follicular structures (other follicular growth inhibited) are steroidogenically active (producing large amounts of estradiol) and are morphologically healthy. When cyst turnover occurs, a new wave of follicular growth is initiated (with recruitment, selection, and dominance of the new wave). Thus, the original dominant cyst has lost dominance (nondominant cyst). Observation of nondominant cysts has shown that they are not steroidogenically active (low levels of estradiol and androstenedione) and have a morphologic appearance similar to that of atretic follicles. Granulosa cell layers have thinned or are missing.[19] Growth of follicles that developed into cysts and follicular dynamics of waves of follicular/cyst growth are therefore similar in many aspects to those in cows with normal estrous cycles.

ETIOLOGY OF CYSTS

Extrafollicular Mechanisms

It was suggested in the early literature that cysts probably result from an excess of secretion of FSH and an insufficient amount of luteinizing hormone (LH). Subsequently, however, two studies from the 1990s showed no difference in serum concentrations of FSH in cows experiencing development and maintenance of cysts and in normal control animals.[10,13] By contrast, mean serum concentrations of LH are nearly twice as high in cows with cysts as in control animals. Pulsatile secretion of LH during cyst growth and development is characterized by high pulse frequency and high pulse amplitude. A preovulatory-like surge of LH, however, is not detected in cows with cysts. Concentrations of LH in serum of cows that spontaneously recover without exogenous treatment are intermediate between those in control animals and those in cows that develop cysts.[10] Infusion of LH at levels that mimic circulating levels in cows with cysts into cycling of postpartum dairy cows did not result in induction of development of cysts.[20] Thus, increased pulsatile secretion of LH alone is not sufficient to induce cysts.

Another characteristic of cows with cysts is the lack of an LH surge when follicles reach ovulatory size.[10,13] In addition, exogenous treatment in cows with cysts with estradiol fails to induce an LH surge in some cows, and the LH surge is delayed in other cows with cysts that did exhibit an LH surge.[21] In two different studies, ablation of follicles followed by an estradiol-induced LH surge with no dominant follicle present was followed by development of large follicles that resembled cysts.[22,23] In those studies, treatment of the cows with cysts with exogenous progesterone was followed by development of a large follicle that ovulated. Thus, cows with cysts appear to be refractory to the stimulatory effects of estradiol on the gonadotropin-releasing hormone (GnRH) surge center; the lack of responsiveness of the GnRH surge center is associated with development of cysts. This observation is consistent with the observation that successful treatments for cysts involve exposure of the cow to progesterone (see later "Treatment" section). Exposure to either exogenous or endogenous progesterone decreases tonic secretion of LH and restores hypothalamic responsiveness to estradiol.

The pituitary gland does not seem to be involved in development of cysts. Pituitary gland content of FSH and LH in cows with cysts is similar to that in cows with normal estrous cycles, and cows with cysts can release LH in response to exogenous GnRH.[1,24]

Molecular Characteristics and Mechanisms

Previous experiments have shown differential expression of messenger RNAs for the gonadotropin receptors and key steroidogenic enzymes during growth of normal follicles at critical stages of development, and expression also has been shown to occur during abnormal follicular development (cysts). In addition, steroid concentrations and content have been shown to be different. Concentration of estradiol (ng/ml) and total content of steroids (ng/follicle) is higher in dominant cysts than in dominant follicles.[2] Messenger RNA expression for the LH receptor in granulosa and theca cells and 3β-HSD in granulosa cells also is elevated in dominant cysts compared with that in dominant follicles. Conversely, nondominant cysts have higher concentrations of progesterone but lower concentrations of estradiol in cyst fluid. In addition, messenger RNA concentrations for the gonadotropin receptors and steroidogenic enzymes, except for cytochrome P-450scc and 3β-hydroxysteroid dehydrogenase (3β-HSD) are nearly undetectable in nondominant cysts. Granulosa and theca cells usually are degenerated in nondominant cysts. Nevertheless, nondominant cysts are still, in general, larger than dominant follicles.

Cysts also have been classified as follicular or luteal. **Follicular cysts** may be single or multiple and are thin-

walled. Follicular cysts with several layers of granulosa cells secrete estradiol (dominant cysts), whereas those with few granulosa cells have low intrafollicular concentrations of estradiol (nondominant). **Luteal cysts** have thickened walls and theca cells, and sometimes granulosa cells are luteinized. Luteal cysts usually secrete progesterone at various levels, sometimes in enough quantity to raise circulating concentrations of progesterone similar to normal luteal phase levels. In some cases, luteal cysts may not be producing significant quantities of progesterone, and circulating concentrations of progesterone may be low. It is believed that luteal cysts develop from follicular cysts when theca and granulosa cells luteinize over time.

DIAGNOSIS OF CYSTS

Early diagnosis of cysts was based on behavioral and physical characteristics. Some cows with cysts expressed nymphomaniac behavior by exhibiting frequent periods of prolonged estrus. It was observed, however, that most cows with cysts did not exhibit estrus. Many long-term anestrous cows had cysts. It is now recognized that greater than 80% of cows with cysts are anestrous. As stated earlier, diagnosis is made on examination of the reproductive tract per rectum by tactile or ultrasound examination based on the presence of one or more structures 2.5 cm in diameter or larger in the absence of a corpus luteum (see Fig. 47-1). As previously mentioned, however, some cysts may be only slightly larger than ovulatory follicles.

Based on the previous description of the dynamic nature of cyst growth dynamics, and the presence of dominant versus nondominant cysts and follicular versus luteal cysts, accurate diagnosis is difficult with use of only a single examination. In addition, diagnosis is more accurate with ultrasonographic examination of the ovaries than with rectal palpation. Early developing corpora lutea, which often are soft to touch, frequently are misdiagnosed as cysts by palpation per rectum. The clinician also should check for pregnancy because some corpora lutea of pregnancy also are soft to tactile examination. Nondominant cysts often are larger than the newly developing cyst. Because nondominant cysts are atretic, they do not respond to exogenous treatment with GnRH or human chorionic gonadotropin (hCG). When nondominant cysts are present, the follicular structure that responds to treatment usually is another follicular structure, sometimes smaller in size than the nondominant cyst. Nondominant cysts often are not greatly reduced in size when a new follicular/cyst wave has proceeded through selection and dominance. Thus, in subsequent examinations, it may be erroneously assumed that treatment was not successful if the nondominant cyst has not decreased in size and the new developing luteal structure is overlooked.

Differentiation of follicular from luteal cysts is not accurate with a single examination by palpation per rectum. Farin and associates report inability to differentiate between follicular and luteal cysts using palpation per rectum, even for experienced clinicians.[25,26] The same clinicians, however, were successful in such differentiation (in approximately 90% of examinations) when using ultrasonographic evaluation. In a few cases, luteal-appearing tissue was detected with low circulating levels of progesterone, and in a few others, circulating progesterone was observed in the absence of thickened cyst walls.

TREATMENT OF CYSTS

Evaluation of treatments is complex because some cysts spontaneously recover, and misdiagnosis is possible. When cysts develop before first ovulation during the postpartum period, up to 50% of the cysts recover spontaneously. If cysts develop after first ovulation, approximately 20% recover spontaneously.[18] Historically, treatment of cysts has included manual rupture and various hormonal regimens. From information provided in the earlier section on etiology, it is evident that successful treatment requires exposure of the central nervous system to endogenous or exogenous progesterone.

Successful treatment of cysts in cows has been with GnRH or with LH or LH-like products (hCG) (see the review by Kesler and Garverick).[18] Both agents induce luteinization, but not ovulation, of cysts and production of progesterone, followed by the development of a dominant follicle that ovulates. After treatment with GnRH or hCG, pulsatile release of LH is greatly decreased. The luteinized cysts produce progesterone for 15 to 18 days, and the cow returns to estrus in about 21 days after GnRH treatment. The progesterone from the luteinized cyst sets up the cascade of events leading to release of $PGF_{2\alpha}$ from the uterus, which induces regression of the luteinized cyst. The interval to the subsequent estrus can be shortened by treatment with $PGF_{2\alpha}$ 9 days after injection of GnRH.[27]

Another regimen also has recently been shown to be effective. Treatment with exogenous progesterone was shown to decrease pulsatile secretion of LH and to induce atresia of cysts and development of an ovulatory follicle.[19,28,29] Thus, successful treatments for cysts have been associated with decreased pulsatile secretion of endogenous LH and an increased secretion of progesterone after treatment, with both endogenous and exogenous sources of progesterone. With exogenous progesterone sources, endogenous LH secretion is reduced, and the hypothalamic-hypophyseal axis "sees" progesterone and can respond to the estradiol produced by the subsequent dominant ovarian follicle by releasing an ovulatory dose of LH. It is probable that a near-luteal level of circulating progesterone is needed to reduce pulsatile LH secretion and sensitize the hypothalamus to respond to the endogenous estradiol produced by the developing follicle.

With GnRH or hCG treatment, LH pulsatility is reduced after treatment, luteinization of the steroidogenically active cyst occurs, and the hypothalamic-hypophyseal axis "sees" progesterone produced by the cyst and can respond to the increasing levels of estradiol from the subsequent dominant follicle to release a preovulatory surge of LH for ovulation. It should be noted that nondominant cysts that have characteristics of atretic follicles do not respond to the GnRH-induced

release of LH or the exogenous LH-like compounds. In such instances, another follicular structure responds to the treatment, or corrective response does not occur. It is important to fully examine the ovaries of cows treated for cysts to determine whether another structure has responded to treatment when the diagnosed cystic structure has not done so.

The recent development of the Ovsynch protocol for timed breeding of large groups of cows in dairy herds also is effective when cows with cysts are included in the breeding program. In this protocol, cows are treated with GnRH, followed 7 days later with $PGF_{2\alpha}$; treatment with another GnRH injection 2 days later; and timed breeding of cows 12 to 16 hours later.[28] Thus, this treatment is similar to that in the study reported earlier whereby the GnRH injection induces luteinization of the cystic structure, which is followed by $PGF_{2\alpha}$-induced luteolysis.[27] The difference is that the interval from GnRH to $PGF_{2\alpha}$ injection is shortened from 9 to 7 days in the Ovsynch protocol.

PREVENTION OF CYSTS

Several studies have reported that cysts are heritable. In one study, the incidence of cysts in Sweden was reduced from 10% to 3% by selection against sires producing daughters that developed cysts.[29] Estimated risk of transmission of the relevant trait, however, typically is small (0.11 to 0.15).[30] Selection against cysts will be slow as a result of the low heritability.

Treatment with GnRH given at days 12 to 14 after parturition reduces the incidence of cysts in the postpartum period.[21,31] Of interest is the finding that cysts do not develop in cows that have a dominant follicle and ovulate in response to the GnRH treatment. This finding indicates that early establishment of estrous cycles during the postpartum period and exposure of cows to luteal levels of progesterone may reduce incidence of cysts in postpartum cows. Nevertheless, attempts to program ovarian function with an intravaginal device during the postpartum period have not reduced the incidence of cyst development.[32]

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CHAPTER 48

Infertility Due to Noninflammatory Abnormalities of the Tubular Reproductive Tract

CHRISTIAN W. STEENHOLDT

Infertility in the cow can be attributed to a number of conditions affecting the tubular reproductive tract. Studies have confirmed that inflammatory disease (e.g., endometritis) is considerably more likely to compromise fertility than is noninflammatory disease.[1,2] In these limited surveys, noninflammatory lesions of the tubular tract accounted for approximately 5% of all lesions detected and were more prevalent among nulliparous animals. As expected, a larger proportion of nulliparous than of multiparous animals will be affected by noninflammatory lesions, in keeping with the absolute state of infertility in many congenital conditions. Although noninflammatory abnormalities are of relatively minimal importance overall, the cases provide for interesting discussion, particularly with regard to mode of development. The embryology of the reproductive system often is a critical factor in the pathogenesis of most such conditions, and the reader is encouraged to review the development of the female tract. This chapter focuses on several conditions that are responsible for infertility due to noninflammatory abnormalities of the tubular reproductive tract.

FREEMARTINISM

Freemartinism is the most commonly recognized noninflammatory condition resulting in infertility involving the tubular reproductive tract in the bovine. Freemartin heifers result from 92% of heterosexual twin births,[3] and it is estimated that at least 86,000 freemartins are born annually in dairy cattle breeds in the United States.[4]

The development of the freemartin heifer is a result of the fusion of the chorioallantoic portions of the twin placentas, which usually is established between days 28 and 30 of gestation and results in a common blood supply between twin fetuses. This common supply allows the exchange of humoral and cellular elements between fetuses, producing calves that are blood cell chimeras. **Chimeras** are individual animals that contain two cell types originating from separate zygotes. Testicular devel-

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opment occurs before ovarian development in the bovine, and anti-müllerian hormone (müllerian-inhibiting substance) from the testes of the male fetus inhibits the development of the paramesonephric (müllerian) ducts in the female.[5] This results in dysfunction in the differentiation of the ovaries, oviducts, uterus, cervix, and cranial vagina. The pathomechanism in 8% of the cases in which a fertile female is born as a twin to a male probably is failure of fusion of fetal membranes or timing of membrane fusion after a critical point in reproductive organ differentiation.[6] The male co-twin usually also is chimeric and may exhibit deficient reproductive capacity.[7] Approximately 6% of twin pregnancies result in the birth of a single calf,[8] and in rare cases a female freemartin calf is born as an apparent singleton as a result of death of the male co-twin in utero.[9] Freemartinism occasionally occurs in conjunction with other congenital abnormalities such as atresia recti[10] and urethral hypoplasia.[11,12]

Clinically, freemartin heifers exhibit broad variation in appearance of the external genitalia, with features ranging from apparent normality (most common) to a small vulva, increased anogenital distance, an enlarged clitoris, and a prominent tuft of hair on the ventral commissure of the vulva. The internal reproductive organs usually are abnormal, with vestigial or masculinization of the ovaries, reduced development of the paramesonephric (müllerian) duct system, and development of the mesonephric (wolffian) duct system. Freemartin females show a range in the internal reproductive organs dependent on the level of masculinization. The least masculinized form is most common, with a small genital tract with hypoplastic ovaries, a short vagina, and an absent cervix. Masculinization appears to be due to in part to a probably indirect role of antimüllerian hormone on gonadal development[13] and the influences of H-Y antigen[14] and results in ovaries with parenchyma that contains tubules and interstitial tissue, resembling testes. In animals that have undergone masculinization, development of the mesonephric (wolffian) ducts also occurs, which may result in epididymides, vasa deferentia, and vesicular glands.[15] It is suggested that the extent of transformation of the female freemartin is dependent on the stage in gestation at which extensive anastomosis exposed her to circulating male hormones.[16] Of interest, the proportion of male blood cells in circulation apparently has no relationship with the degree of masculinization in freemartin heifers.[17]

The diagnosis of freemartin heifers usually is based on a history of heterosexual multiple births, appearance of the external genitalia, and palpation or ultrasound evaluation of the internal reproductive tract. Visualization via vaginal speculum usually will reveal a short vagina and no external cervical os in freemartin heifers. The vagina in suspected freemartin heifers may be probed using a suitable instrument (a test tube, insemination pipette, or thermometer is commonly used); alternatively, commercial probes are available (Ru-an Freemartin Probe, $US25.95, Valleyvet.com). Freemartin heifers generally have a vagina that is approximately one-third the length of vaginas in normal heifers of the same age. Among heifers younger than 1 month of age, the freemartins usually will have a vagina length of 5 to 6 cm, whereas in normal heifers, vaginas are 13 to 15 cm long.[18,19] The vaginal length in suspected freemartin heifers should be compared with that in normal heifers of the same size. Occasional false positives using the vaginal length test can be due to interference by a persistent hymen with the deposition of the probe. False negatives also may be encountered, because some freemartins have a vagina that is of normal length. Eighty percent of freemartin heifers will be correctly identified using a vaginal probe,[20,21] with the remaining 20% of heifers likely to consist of approximately half freemartins and half fertile heifers. It is suggested that laboratory testing be carried out on the heterosexual twin heifers with normal vaginal length.

Laboratory testing includes polymerase chain reaction (PCR) analysis, karyotyping, blood typing, and the erythrocyte lysis test for the detection of blood cell chimeras. Although most female cattle that are blood cell chimeras are likely to be sterile freemartins, several fertile XX/XY animals have been described.[22–25]

PCR assay has become the most common commercially available test to diagnose freemartins. The PCR procedure demonstrates sex chromosomes exhibiting XY and XX in the same animal. The test is relatively fast and highly accurate because it can detect the presence of as little as 1 in 10,000 blood cells containing the Y chromosome.[26] A blood tube containing 5 to 8 ml of whole blood with ethylenediaminetetra-acetic acid (EDTA) or a drop of blood on a blood card from a suspected freemartin may be submitted for testing. The cost for one assay in 2005 was $US40. This test is available from several laboratories including Biogenetic Services Inc., Brookings, SD (info@biogeneticservices.com), and Veterinary Genetics Laboratory, Davis, CA (cattle@vgl.ucdavis.edu).

Karyotyping is another method for demonstrating blood cell chimerism. Blood lymphocytes are cultured and metaphase chromosome spreads are examined for XY cells. The distribution of male cell percentages in nucleated blood cells appears to be random in individual animals, and freemartins may contain a high to low percentage of XY cells.[27] False negative results can occur in animals containing a small proportion of XY cells if insufficient metaphases are counted. Karyotyping requires considerable laboratory work and is fairly expensive. Another interesting test of XX/XY chimerism is through skin grafting between heterosexual twins—freemartin heifers accept a surgically placed skin graft from their male twin.[28]

OTHER INTERSEX CONDITIONS RESEMBLING FREEMARTINISM

Intersex cattle other than freemartins are very rare. Male pseudohermaphrodites are genetic and gonadal males, but the external genitalia resemble those of a female. Male pseudohermaphrodites are more common than female or true hermaphrodites, probably because more genes are required to initiate male development (steroidogenic enzymes, 5α–reductase, and androgen receptors), with correspondingly greater opportunity for genetic

defects. Limited case reports of male pseudohermaphrodites in cattle have been published.[29–32] Classification of intersex cases requires careful anatomic (often at post mortem), chromosomal, and endocrinologic examination. It is possible that some other intersex conditions are misclassified as freemartins if the diagnosis is based solely on physical examination.[33]

Bovine male pseudohermaphroditism (often referred to as testicular feminization) results from androgen insensitivity. Affected animals are XY males with testes, paramesonephric and mesonephric (wolffian) duct regression, and female-like external genitalia. The tubular genitalia of the paramesonephric (müllerian) system are underdeveloped owing to testicular production of antimüllerian hormone (müllerian-inhibiting substance). Production of testosterone by the testes is normal, but because of intracellular androgen insensitivity, the mesonephric (wolffian) system fails to develop without androgenic support. It is possible that this disorder is inherited as an X-linked trait in cattle, as in other species.[31] Clinically, affected animals may be mistaken for heifers but fail to show estrus and exhibit bull-like behavior. On examination of the internal reproductive tract, the vagina may be short to normal in length; no cervix is present, with a very small or absent uterus and testes in the normal position of ovaries.[32]

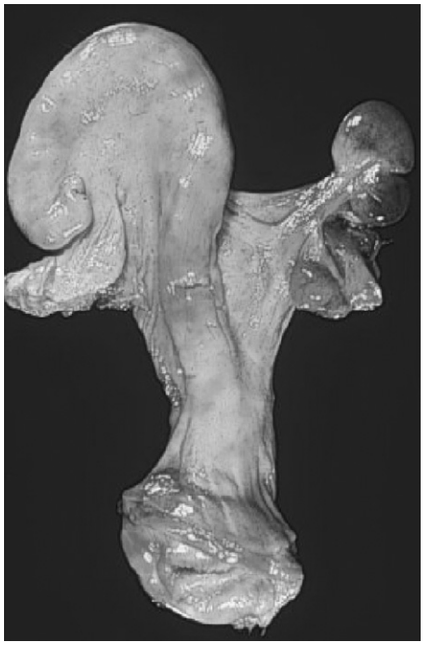

Fig. 48-1 Segmental aplasia affecting the right uterine horn. (Courtesy of Dr. C.D. Buergelt, University of Florida.)

SEGMENTAL APLASIA OF THE PARAMESONEPHRIC (MÜLLERIAN) DUCTS

Segmental aplasia is a result of a prenatal lack of development of a portion of the paramesonephric (müllerian) duct system, resulting in various degrees of aplasia, potentially involving the vagina, cervix, uterus, and oviducts. Historically described as "white heifer disease"[34,35] owing to a high prevalence in white Shorthorn females, segmental aplasia has been reported in several other breeds of cattle including Holsteins,[1,36] Senepol,[37] German Black Pied,[38] Guernsey,[1] Jersey,[1] Angus,[39] and Ayrshire.[39] The prevalence of segmental aplasia ranges from 0.15%[1] to 0.2%[40] in slaughterhouse studies. Although rarely diagnosed in practice, segmental aplasia in cattle provides an often academic discussion of the locally acting nature of endometrial prostaglandins, the likely inheritance of the condition, and cull versus treatment options.

A range of defects of the paramesonephric (müllerian) system may be seen in animals affected by segmental aplasia. Individual cattle may present with abnormalities ranging from an imperforate hymen to complete maldevelopment of the tubular reproductive tract. The most common presentation encountered in practice is aplasia and resultant blockage affecting the caudal portion of one uterine horn, often referred to as **uterus unicornis**, resulting in accumulation of endometrial secretions in the cranial horn (Fig. 48-1). In a review of 20 cases of uterus unicornis, 14 animals had a defective right uterine horn and 6 had a defective left horn.[39] A persistent hymen usually is not present.[39] When both horns are affected, the aplasia includes the cervix and vagina.[41] Segmental aplasia of the oviducts is rare,[2] and obstruction of the oviducts may result in accumulation of secretions and hydrosalpinx. The aplastic section of the reproductive tract usually consists of a band of connective tissue and muscle with no lumen, mucosal epithelium, or glands.[36,41]

A **persistent hymen** is considered to be a form of segmental aplasia and is a result of an embryonic malunion of the paramesonephric (müllerian) ducts and the ectodermal urogenital sinus. Heifers that have a complete hymen accumulate secretions in the vagina and also may develop mucometra. Tenesmus is an occasional presentation and is due to significant fluid accumulation in the cranial vagina. A persistent hymen occurs occasionally in the cow, either as a single lesion or associated with segmental aplasia.[42]

Fluid accumulation in cases of segmental aplasia may be misdiagnosed as pregnancy, pyometra, mucometra, or cystic ovarian disease. Rarely, retained endometrial secretions become inspissated and surrounded by oily fluid and may be confused with a fetal mummy.[43] Diagnosis of segmental aplasia usually is achieved through careful rectal palpation or transrectal ultrasound examination and identification of the abnormal anatomy as indicated by the inability to pass a pipette into both uterine horns. A definitive diagnosis is achieved at necropsy examination.

In the Shorthorn breed and derivatives (e.g., Belgian Blue), inheritance appears to be linked to the roaning gene.[44] A high incidence of segmental aplasia, causing a reduction in herd fertility, has been reported[45,46] in association with the extensive use of carrier sires. In the Holstein breed, inheritance is suggested to be due to autosomal recessive genes that are linked for aplasia affecting the left and the right uterine horns.[41] Anecdotally, some Holstein cows may not pass on the trait to offspring (Strelow L: personal communication, 2005).

Segmental aplasia affecting one uterine horn provides a natural model for locally acting hormonal control by the endometrium on the ipsilateral corpus luteum. Animals with segmental aplasia affecting one uterine horn show erratic estrus intervals[47] or anestrus[36] as a result of failure of local endogenous prostaglandin F_{2a} production from minimal or damaged endometrium.

Infertility is a likely presentation in affected animals, although presentation for pregnancy diagnosis also is not uncommon. Therapy of affected animals should be tempered with the recognition of the likelihood of potentiation of the condition, particularly in breeds in which a genetic mode of transmission has been clearly defined. Treatment modalities in animals with one aplastic uterine horn have included insemination at detected estrus, palpation or ultrasound examination to detect the side of ovulation and subsequent CL formation, and short-cycling the cow with prostaglandins until the animal ovulates of the ovary on the normal horn. Ovariectomy via laparotomy or colpotomy[48] of the ovary ipsilateral to the affected horn, thereby ensuring that ovulation occurs only on the unaffected side, is another potential treatment option. Affected animals may maintain pregnancy through use of embryo transfer even if the cow ovulates on the affected side through the administration of human chorionic gonadotropin (hCG) every 10 days, starting 3 days after embryo transfer until an accessory CL is created on the unaffected horn. Some cows affected with segmental aplasia have made successful embryo donors, with unaffected offspring (Strelow L: personal communication, 2005). Heifers that have a persistent hymen as the only abnormality may be treated by creation of a cruciform incision in the hymenal tissue.

NEOPLASIA

Neoplasia affecting the reproductive tract is considered rare in cattle. Most reported cases have been diagnosed in abattoirs at postmortem meat inspection, and a clinical history is rarely available. Tumors that occupy a large amount of the uterine wall or occlude the uterine lumen may result in reduced fertility or abortion.[49,50] The most prevalent tumor type affecting the tubular reproductive tract differs among published series. Rebhun[49] reports that lymphosarcoma is the most common tumor affecting the uterus. Noakes and associates[51] and Roberts[39] suggest benign tumors of the mesenchymal tissues (e.g., leiomyoma, fibromyoma, fibroma) to be most common. In view of the high seroprevalence of BLV in North American cattle[52] and the considerable number of condemned carcasses as a result of systemic lymphosarcoma,[53] it would be expected that this tumor type would be predominant in the United States and Canada. In regions with low seroprevalence of BLV (e.g., Europe, Australasia),[54] it is likely that the benign tumor types would be more prevalent.

Considerations in the differential diagnosis for neoplastic conditions of the tubular reproductive tract include pregnancy (placentomes or fetus), abscess, adhesions, mummified/macerated fetuses, fat necrosis, and tuberculosis. Depending on the tumor type, neoplasia affecting the uterus may appear as singular discrete masses, numerous masses, or thickening of the uterine wall. Leiomyomas are smooth-surfaced, discrete, benign tumors that consist of smooth muscle fibers and collagen and resemble smooth muscle on cut surface. Leiomyosarcomas are locally invasive and may contain areas of necrosis. Fibromas are hard, white, round masses that consist of dense bundles of collagenous fibrous tissue.[55] Adenocarcinomas arise from the uterine wall and do not usually affect mucosal and serosal surfaces. When cut, this tumor type is hard (calcification) and appears white to yellow, and metastases commonly are found in the internal iliac lymph nodes and lungs.[55] Lymphosarcoma can be focal, multifocal, or diffuse, with local lymph nodes usually also affected. Cows that are pregnant and affected by uterine lymphosarcoma typically produce small, nonviable calves.[49] Lymphosarcoma, adenosarcoma, and carcinoma in the reproductive tract may be primary but often tend to be metastatic from a distant site. Definitive diagnosis may be achieved through cytologic examination after biopsy or at postmortem examination.

Partial hysterectomy via flank laparotomy may be considered for management of neoplastic conditions affecting one uterine horn. Leiomyomas and fibromas are the uterine neoplasms most amenable to surgical resection. Early leiomyosarcoma also can be successfully resected if removed before metastasis occurs. Surgical approaches through a ventral midline, caudal flank, or ventrolateral incision have been described.[56,57] The affected uterine horn is exteriorized, and the ovarian pedicle is double-ligated using absorbable suture material and then transected. The broad ligament is dissected from the uterine horn to the site that is most suited for transection (usually the base of the affected horn). The affected horn is removed, and the stump is oversewn with a double-layered inverting suture pattern. Closure of the abdomen is routine. Animals are considered to be suitable surgical candidates if the neoplastic portion of the uterine horn is located cranially, allowing adequate closure of the stump, so as not to affect the patency of the nonaffected horn. Cows have maintained pregnancy after removal of one uterine horn.[57]

INCOMPLETE FUSION OF THE PARAMESONEPHRIC (MÜLLERIAN) DUCTS

Failure of complete fusion of the paramesonephric (müllerian) ducts during reproductive tract development results in a range of abnormalities, mostly affecting the cervix. The most common presentation of this disorder is the development of a double external cervical os (Fig. 48-2). Mild cases result in a vertical band of tissue caudal to the cervix that has little effect on reproduction. Both external openings may join into a normal internal cervical os, or one side may end in a blind diverticulum. This abnormality often goes undetected but occasionally may cause difficulty in passing an artificial insemination pipette or result in dystocia when parts of the calf pass each side of the two external cervical canals. Fertility is reported as normal[39] to subfertile[58] in affected animals. Studies have reported an incidence of between 0.2%[1] and 12.8%[59] of cows with a double cervix. A high incidence

Fig. 48-2 Double external cervical os. (Courtesy of Dr. C.D. Buergelt, University of Florida.)

of this condition has been found in some herds[59] and breeds,[57] but the general prevalence is likely to be low. Inheritance is reported to be due to an autosomal dominant gene with incomplete penetrance[58] or a single autosomal recessive gene with low penetrance and variable expressivity.[59]

The most severe manifestation of the condition is **uterus didelphys**, which involves complete failure of fusion of the paramesonephric (müllerian) ducts, resulting in separation of the uterine horns and a complete double cervix, with a band of tissue extending to the vaginal vestibular junction.[60] Fertility is affected, because semen must be deposited in the cervical os that is ipsilateral to the ovulating ovary. Reports indicate that cows may become pregnant,[61] but on account of the division of the uterine horns, placental attachment in the nongravid horn is impossible, and the pregnancy may not be carried to term.[62]

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CHAPTER 49

Bacterial Causes of Bovine Infertility and Abortion

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Bacteria generally are cited as the most common agents of infection causing abortion in cattle. In large retrospective studies, bacterial infections of the fetoplacental unit were identified in 15% to 16.2% of aborted bovines and accounted for 48% to 58% of abortions caused by infectious agents. Abortion in cattle has been associated with more than 25 different species of bacteria, which vary considerably in their pathogenicity for livestock (Table 49-1). It appears that if a bacterium can survive transit in the maternal bloodstream to reach the fetoplacental unit, it has the potential to cause abortion. The fetus may be particularly susceptible to infection by a wide variety of organisms as a result of its immature immune system and suppression of the immune response at the junction of the maternal and fetal placenta.

Diagnostic laboratory data have provided valuable insight into the relative prevalence of bacterial species that contribute to the development of bovine abortion. Unfortunately, similar information is not available for bacterial causes of infertility. *Campylobacter fetus* spp. *veneralis, Leptospira*, *Mycoplasma, Ureaplasma, Chlamydia,* and *Haemophilus somnus* all have been associated with bovine infertility, but at present the relative prevalence and importance of these agents are unknown.

A number of the bacterial causes of bovine abortion and infertility are potential zoonoses. The agents of these diseases include *Leptospira, Brucella, Listeria, Chlamydia, Salmonella,* and *Campylobacter* spp. Care should be taken in handling and transporting the uterine discharge or products of abortion, to minimize or eliminate opportunities for human exposure.

BOVINE ABORTION CAUSED BY OPPORTUNISTIC BACTERIA

By far the larger portion of this chapter discusses the specific agents recognized as potential causes of bovine abortion. The vast majority of bacterial abortions in cattle (82%), however, are caused by organisms generally considered to be opportunists. These opportunistic organisms fall broadly into two overlapping categories: (1) bacteria that are part of the normal microflora of the mucosal surfaces (*Arcanobacterium pyogenes, Pasteurella multocida, Mannheimia hemolytica, Haemophilus somnus, Escherichia coli, Campylobacter* spp., *Staphylococcus* spp. *Streptococcus* spp.), and (2) common environmental bacteria (*Bacillus* spp., *Pseudomonas* sp, *E. coli*). These ubiquitous bacteria occasionally are able to enter the maternal bloodstream, survive transit to the fetoplacental unit, and cause abortion. Such agents are not considered to be contagious or transmissible causes of abortion.

The significance of isolating one of these agents from an aborted bovine conceptus depends on the incidence of abortion and the clinical signs in the herd. Recovery of any of the aforementioned organisms in isolated abortions generally is of minimal significance from a herd health standpoint. Isolation of one or more species of opportunistic bacteria from fetuses during an outbreak or ongoing abortion problem suggests that (1) access of these ubiquitous organisms to the maternal bloodstream has been enhanced or (2) abortions are a consequence of another disease process.

Although difficult to determine with certainty in retrospect, the occurrence of abortions may be a function of factors that enhance access of ubiquitous bacteria to the maternal bloodstream. Such factors could include subclinical or clinical acidosis (rumenitis), external lesions, or poor feed quality leading to microtrauma in the upper digestive tract. Anecdotal information from diagnostic laboratory submissions would support a link between subclinical or clinical acidosis and bacterial abortion in both dairy and beef cattle.

Pathogens of adult animals, such as *Salmonella, Mannheimia hemolytica, H. somnus,* and *P. multocida,* can cause abortion in one of two ways. With the exception of *Salmonella,* these organisms are ubiquitous members of the normal microflora and can behave as opportunists. They also may cause abortion as a result of disease processes in the adult, in which case abortion is a secondary consequence of the organism's ability to cause enteric or respiratory disease, with bacteremia leading to fetal infection. Although these agents can be considered contagious pathogens, they typically are not classified as contagious causes of abortion.

Diagnosis

Diagnosis is based on the isolation of a moderate to heavy and relatively pure growth of a bacterium from fetal tissues and/or placenta in conjunction with confirmatory gross and microscopic lesions. The four tissues from which bacteria are most commonly isolated are placenta, abomasal contents, lung, and liver. Culture of placenta is somewhat problematic because it often is contaminated by bacteria from the vaginal or vulvar region or the environment. Nonetheless, it is imperative to submit placenta for culture because the infectious process may be con-

Table **49-1**

Bacterial Agents Associated with Abortion or Stillbirth in Cows*

Agent	Frequency (% of total bacteria isolated)
Arcanobacterium pyogenes	29.2
Bacillus spp.	24.8
Listeria	9.4
Escherichia coli	7.6
Leptospira	6.1
Mannheimia hemolytica	2.9
Streptococcus spp.	2.1
Pasteurella multocida	2.0
Salmonella spp.	2.0
Brucella abortus	1.9
Haemophilus somnus	1.6
Staphylococcus spp.	1.6
Campylobacter fetus spp. *veneralis*	1.5
Campylobacter fetus spp. *fetus*	1.3
Pseudomonas spp.	1.2

*Bacterial species encountered in 8962 bovine abortions or stillbirths. Data from Kirkbride CA: Bacterial agents detected in a 10-year study of bovine abortions and stillbirths. *J Vet Diagn Invest* 1993;5:64.

fined to the placenta, so that the organism will not be recovered from fetal tissues or fluids. Gross lesions that may be observed in association with abortions due to opportunistic bacteria include placentitis, fine strands of fibrin covering the viscera, and occasionally epicarditis (in abortion due to *Bacillus* spp.). Microscopic lesions typically include changes characteristic of a placentitis, suppurative fetal bronchopneumonia, and occasionally inflammation of the liver.

Treatment and Control

Control of multiple abortions due to opportunistic bacteria is based on diminishing opportunities for ubiquitous organisms to gain access to the maternal bloodstream and survive transit to the fetus. This is accomplished by optimizing the health status of the cow (nutrition, stress, environment) and addressing factors associated with enhanced access of these bacteria to the maternal bloodstream, such as acidosis, poor feed quality, and presence of external lesions. Control of abortions that occur as a consequence of an infectious process in the dam is based on controlling the underlying disease process in the cow.

BOVINE ABORTION AND INFERTILITY RELATED TO INFECTION BY SPECIFIC PATHOGENS

Brucellosis

Brucellosis once was considered to be the most important reproductive disease of cattle. Today, however, the State-Federal Brucellosis Cooperative Eradication Program, launched in 1934, as well as subsequent programs, has resulted in the elimination of this disease from commercial herds in the Unites States. As of December 2000, no known commercial cattle or bison herds were infected with *Brucella* in the United States. Currently the only remaining focus of brucellosis in the United States is in the Greater Yellowstone Area, which includes Yellowstone National Park, Grand Teton National Park, the National Elk Refuge, and portions of Idaho, Montana, and Wyoming, where free-ranging bison and elk are infected. These animals pose a disease threat to cattle in surrounding brucellosis-free areas and serve as a potential source for disease reintroduction into domestic cattle and bison herds. Transmission from bison to cattle has been demonstrated when *Brucella*-infected bison were penned with unvaccinated, seronegative cattle. Documented cases of natural transmission of *Brucella* from bison or elk to cattle have not been reported, however.

Currently, 46 states, Puerto Rico, and the U.S. Virgin Islands are classified as brucellosis-free, meaning that they have had no infected cattle or domestic bison herds for at least 1 year. Four states—Florida, Missouri, Oklahoma, and Texas–are close to complete eradication of the disease and, with no known infection at this time, are now in the final 1-year countdown phase.

Continued surveillance is necessary to prevent this insidious disease from once again gaining a foothold in U.S. domestic populations. It has been estimated that if brucellosis were allowed to spread, beef and dairy production costs would increase by $80 million within 10 years.

Agent, Source, and Epidemiology

Brucella abortus is a small, nonmotile, nonsporulating, nonencapsulated, gram-negative coccobacillus. Brucellae are facultative intracellular pathogens that are able to survive and multiply within phagocytic cells and lymphoid tissues as a result of their ability to prevent fusion of lysosomes with the phagosome.

The infected cow is the principal source of *Brucella.* The aborted fetus, placenta, and uterine discharge contain large numbers of organisms. Transmission typically is the result of ingestion of the products of a *Brucella*-induced abortion (fetus, placenta, uterine discharge) or ingestion of material contaminated by these products. *Brucella* may be present in uterine discharges beginning 2 weeks before calving or abortion, and lasting 2 to 3 weeks thereafter. The incubation period is quite variable, ranging from 2 weeks to 1 year or longer. The minimum incubation period from infection to abortion is approximately 30 days.

In the bull, *B. abortus* infection may produce orchitis, epididymitis, and inflammation of the accessory reproductive organs. Orchitis may lead to reduced libido and spermatogenesis and impaired fertility. Although the organism can be transmitted in semen, the bull appears to play a minor role in the transmission of *Brucella.*

Pathogenesis

Brucella penetrates the mucosa of the nasal or oral cavities after ingestion. *B. abortus* initially localizes in lymph nodes, infects the gravid uterus during bacteremia, and multiplies to massive numbers in chorioallantoic tro-

phoblasts, leading to trophoblast necrosis and chorioallantoic ulceration. Fetal bacteremia follows replication of *Brucella* organisms in trophoblasts. Once infection is established in sexually mature animals, it tends to persist indefinitely. Not all infections result in abortion, however, and less than 20% of infected cows abort more than once.

Intracellular replication in chorioallantoic trophoblasts is responsible for the massive accumulation of *B. abortus* in the placenta. The growth of *Brucella* organisms in trophoblasts may be enhanced by their erythritol and/or hormone content, because both erythritol and progesterone augment the growth of *B. abortus* in vitro.

Clinical Signs

The primary sign of brucellosis is abortion. Abortions generally occur after the fifth month of gestation. Retained placenta and metritis are frequent sequelae to abortion. *Brucella*-infected cows also may give birth to weak calves that die shortly thereafter.

Pathology

The most consistent lesion observed in the conceptus is placentitis. In severe cases, the intercotyledonary placenta may be dry, thickened, and cracked and has been described as having the appearance of Moroccan leather. The intercotyledonary placenta may be covered by a thick, yellowish exudate. Cotyledons may demonstrate foci of necrosis and be covered by an exudate. Fetal lungs occasionally are enlarged, firm on palpation, and covered by fine strands of fibrin.

Diagnosis

A definitive diagnosis of *Brucella* abortion requires isolation of the organism from fetal tissues or uterine discharge. The organism can be readily isolated from fetal abomasal contents, lung, placenta, and uterine fluids. It also may be recovered from colostrum, milk, and meconium. In the face of suspicious lesions and negative culture results, immunohistochemistry (IHC) studies can be used to confirm *Brucella* infection. After a diagnosis of *Brucella* abortion, additional infected animals generally are identified by serologic testing. Such testing may not identify all infected animals, because up to 15% of infected cows do not seroconvert until after abortion or calving.

A new systemic vaccine for brucellosis, Strain RB51, has been approved for use in cattle. The advantage of this vaccine is that it induces a protective response without stimulating the development of antibodies that react with standard assays based on *B. abortus* LPS oligosaccharide side chains.

Treatment and Control

Control of brucellosis traditionally has been based on vaccination of calves to reduce the population at risk, control of cattle movement to diminish the spread of disease, and testing to identify reactors. Currently, treatment has no role in the control of bovine brucellosis. Brucellosis is a reportable disease, and regulations require herd quarantine and elimination of all reactors. The current focus of brucellosis control is on surveillance, with rapid elimination of new *Brucella* infections.

Campylobacter fetus spp. *veneralis* Infection

Bovine genital campylobacteriosis is a sexually transmitted disease caused by the gram-negative, microaerophilic rod *Campylobacter fetus* subsp. *veneralis*. This organism is an obligate parasite of the bovine reproductive tract. Bulls are asymptomatic carriers. The clinical effects of infection are manifested in the cow. Historically, campylobacteriosis has been one of the most important sexually transmitted diseases of cattle. Economic losses are the result of poor conception rates, increased culling due to infertility, decreased average weaning weights, and increased management costs related to prolongation of the calving season.

Agent, Source, and Epidemiology

Campylobacter fetus spp. *veneralis* is an extracellular, motile, gram-negative, microaerophilic rod. Cattle constitute the primary host and main reservoir for this organism. Reproductive tract infection in cows and young bulls typically is transient. Bulls younger than 3 years of age tend to be resistant to infection and clear the organism within a few weeks. Mature bulls (4 years of age and older) become chronic carriers.

The disease is transmitted by coitus. The transmission rate from infected bulls to susceptible cows may approach 100%. Infected bulls may carry the organism in the preputial cavity indefinitely. Bulls do not become permanent carriers until they are at least 4 years of age, however, and most not until 5 to 6 years of age. The development of epithelial crypts in the penile mucosa with advancing age provides a favorable habitat for the bacteria. Because infection in young bulls is transient, transmission by these animals relies on sexual contact with an infected cow. Bull-to-bull transmission can occur from contaminated semen collection equipment or through mounting activity when bulls are held in common areas.

Cows become infected after natural service by an infected bull or after insemination with contaminated semen. Infection may be spread from cow to cow through the use of poorly sanitized instruments used for reproductive procedures. Infected cows develop immunity and generally clear the organism within 3 to 6 months of infection, and in a majority of cows, *Campylobacter* will not survive a normal gestation. Some persistently infected cows may harbor the organism for over a year, however, and it has been recovered from cows as long as 196 days beyond the end of pregnancy initiated by infected semen. Failure to eliminate the infection may be due in part to the organism's ability to undergo substantial antigenic change during the course of natural infection. After clearance of infection, cows are resistant to reinfection for a short period of time.

Pathogenesis

The disease is transmitted by coitus. After exposure, the anterior vagina and cervix are colonized, and the infection spreads to the uterus and oviducts within 12 to 14

days. Infection typically does not interfere with fertilization and early embryonic development. Uterine infection leads to early embryonic death as a result of the inflammatory response in the uterus and oviducts. The clinical manifestations of infection in the female seem to depend on the number of organisms contained in the original infective dose and on the rate of multiplication within the uterus. Rapid replication occurs most commonly, causing death of the developing embryo or fetus on days 15 to 80. Because embryonic death typically takes place after maternal recognition of pregnancy (days 15–17), the dam's return to estrus will be delayed. Less rapid replication leads to midterm abortion. *Campylobacter fetus* spp. *veneralis* is progressively eliminated from the oviducts and uterus, and fertility returns. Frequently, however, the organism persists in the cervix and vagina for several more months, during which time the cow remains a source of infection.

Clinical Signs

Detection of clinical signs is dependent on how closely the herd is monitored. Although rarely observed, infection is associated with vaginitis, cervicitis, and endometritis. In closely monitored herds, an increased number of repeat breeders will be identified. The hallmark of *Campylobacter* infection is irregular and delayed returns to estrus. "Infertility" is actually the result of early embryonic death. Herd pregnancy rates will be low (40%–70%), and a wide range of gestational ages may be found at the time of pregnancy examination. Less than 10% of infected cows will abort a detectable fetus. Cows typically abort between the fourth and seventh months of gestation. In *Campylobacter*-endemic herds, clinical signs are most commonly seen in young cows or newly introduced animals.

Pathology

We emphasize that this organism does not cause specific gross lesions in the aborted fetus or placenta. A history of infertility accompanied by a low number of midterm abortions is more indicative of the potential for *Campylobacter* abortion than of gross lesions. Aborted fetuses typically have a placentitis, which is consistently identified microscopically, but gross evidence for a placentitis is variable and often inconspicuous. Changes characteristic of a suppurative fetal pneumonia and hepatitis often are observed on microscopic examination of fetal tissues.

Diagnosis

Diagnosis is based on (1) isolation of the organism, (2) demonstration of the agent in fetal tissues, preputial scrapings, or vaginal mucus with direct fluorescent antibody (FA) tests, or (3) detection of antibodies in vaginal mucus by an enzyme-linked immunosorbent assay (ELISA) or agglutination tests.

Infertility. A definitive diagnosis typically is based on isolation of the organism under microaerophilic conditions on selective media. Culture of preputial scrapings tends to be more productive than that of preputial washes because the organism resides deep in the preputial crypts. Culture of vaginal mucus is most useful when samples are obtained early in the course of infection and when the cows are in estrus. The recovery rate drops substantially in chronically infected cows.

Proper handling of samples is necessary to optimize organism recovery. A transport medium should be used, such as Clark's transport media or Weybridge TEM. Practitioners should contact their regional diagnostic laboratory for media and specific transport recommendations.

Serologic diagnosis of campylobacteriosis is not practical because infection does not stimulate the production of sufficient serum antibody levels to be accurately measured by commercial methods. Genital tract infection in the cow, however, stimulates local immunity, which can be detected by the vaginal mucus agglutination test or an ELISA measuring immunoglobulin A (IgA) antibodies. Both of these tests are used most appropriately on a herd rather than individual animal basis. An indirect FA assay may be used to detect the organism in preputial scrapings from bulls.

Abortion. A rapid, presumptive diagnosis can be made by the identification of small, motile organisms with rapid, darting movement on darkfield examination of stomach contents. This is a screening test for *Campylobacter* and does not differentiate *C. fetus* spp. *veneralis* from *C. fetus* spp. *fetus* or *Campylobacter jejuni*. A definitive diagnosis is based on isolation of the organism from placenta or fetal lung or stomach contents. Metabolic profiles are used to differentiate the various *Campylobacter* species that can be isolated from aborted bovines.

Treatment and Control

Control is based on the following factors: (1) transmission is venereal, (2) older bulls become permanently infected, (3) infected cows typically become immune 3 to 6 months after infection, and (4) vaccination of cows and bulls is both prophylactic and curative.

In uninfected herds, the following measures can help prevent introduction of the disease:

Avoid open range with mixing of cattle.
Avoid leasing/purchasing mature bulls unless they have been thoroughly tested for venereal diseases.
Keep fences in good repair to prevent introduction of unwanted animals.
Restrict replacement animals to virgin bulls and virgin heifers.

> Cows in the late stages of gestation or unbred cows with young calves at their sides are unlikely to be infected. Because of the financial ramifications of introducing this disease into a naive population, vaccination is recommended when the risk of disease introduction is increased.

Once the disease is diagnosed, control is based on identifying and eliminating chronic sources of infection and preventing reinfection. Bulls are the primary source of chronic infection. Blood culture for *Campylobacter* is indicated for all nonvirgin bulls 6 to 8 weeks before the breeding season, and all seropositive animals should be culled. For valuable bulls, treatment is an option. In young bulls, vaccination and sexual rest typically result in elimination of the organism within a few weeks to several months. Therapeutic immunization can extend

the age of natural resistance in young bulls and aid in eliminating infection in bulls up to 5 years of age. Older, chronically infected bulls may be treated. A topical ointment containing 10 g neomycin and 4 g erythromycin in 200 g of carbowax has been reported to be effective when applied to a thoroughly cleaned and exteriorized penis and prepuce for three treatments repeated at 24-hour intervals.

A reliable vaccine is available for campylobacteriosis, and vaccination of cows and bulls can be used for both prevention and elimination of this disease. A unique feature of immunization against *C. fetus* spp. *veneralis* is that it affords dramatic protection against an extracellular, noninvasive pathogen of the genital mucosa—an unusual feature of vaccination. Vaccination against this organism, however, results in both protection against subsequent infection and cure of chronic infection in bulls up to 5 years of age and in cows. In infected herds, all breeding stock, including bulls, should be vaccinated. Animals are initially vaccinated twice, 2 to 4 weeks apart and at least 30 days before start of the breeding season, followed by annual vaccination.

Because campylobacteriosis is a venereally transmitted disease, cessation of natural breeding and the use of artificial insemination techniques for at least two breeding seasons after infection is detected, with appropriate precautions including addition of antibiotics to semen extender and careful sanitation between animals, has been used to eliminate herd infections.

Haemophilus somnus Infection

Haemophilus somnus has been associated with a complex of diseases including thromboembolic meningoencephalitis, polyarthritis, pneumonia, myocarditis, and infections of the reproductive tract. The organism is a common inhabitant of the bovine vagina and is an important cause of vaginitis and endometritis.

H. somnus has been isolated from field cases of abortion, and abortion has been experimentally reproduced at all stages of gestation with the organism. Available evidence, however, indicates that *H. somnus* is not a major cause of abortion in cattle. *H. somnus* was not recovered from aborted fetuses in two large retrospective studies and was identified in only 0.23% of aborted bovines in a third. Documented outbreaks of *H. somnus* abortion are rare, and a majority of abortions due to this agent appear to be sporadic.

The status of *H. somnus* as a cause of infertility is controversial. Studies had demonstrated that *H. somnus* can adhere to the zona pellucida of intact embryos and cause degeneration. These studies, along with epidemiologic information, suggest that *H. somnus* may be capable of causing infertility. Conversely, additional epidemiologic investigations have discounted the ability of *H. somnus* to cause infertility, and attempts to experimentally reproduce infertility with *H. somnus* have not been successful. Because of common presence of this organism in the vaginal or vulvar region and its ability to cause vaginitis/metritis, *H. somnus* is in an ideal position to contribute to infertility. Its association with herd infertility problems, however, has been tenuous.

Agent, Source, and Epidemiology

H. somnus is a gram-negative, non–spore-forming coccobacillus. The bovine reproductive tract is the likely reservoir for this organism because it is a common component of the normal bacterial flora of the male and female genital tract. A 80% to 90% genital carrier rate has been reported in bulls. Except for infrequent reports of infertility and poor semen quality, the organism does not seem to cause significant reproductive disease in bulls. *H. somnus* has been isolated from the genital tract of up to 57% of healthy cows. The organism can remain in the vagina for long periods without clinical signs. Organisms are shed in urine or discharges to contaminate the environment.

Spread to other animals appears to be the result of contamination of the environment by respiratory or vaginal discharges and urine. Reports indicate that carrier bulls can infect cows, and the organism is readily spread by natural breeding.

Pathogenesis

Infertility probably is the result of the effect of *H. somnus* on the developing embryo. As noted earlier, *H. somnus* can adhere to the zona pellucida of intact embryos and cause degeneration. It was able to induce detrimental effects on bovine embryos without causing significant uterine lesions.

Abortion due to *H. somnus* has been experimentally reproduced by intravenous, intratracheal, and intra-amniotic inoculation of the organism. Abortion probably is secondary to hematogenous dissemination following vaginal or respiratory infection. Laboratory studies do not support ascending infection through the cervix.

Pathology

Recognizable gross lesions in the conceptus generally are confined to the placenta and consist of a necrotizing to necrosuppurative placentitis with associated placental edema. Reports indicate that *H. somnus* has the same affinity for the vascular system in the fetus as in the adult, and microscopic examination of placenta and fetus generally will reveal vasculitic changes.

Diagnosis

Infertility. Caution must be exercised when *H. somnus* is considered as a cause of infertility. Although isolation of the organism is essential to confirm a diagnosis, its mere presence in the vagina does not signify clinical disease. A consistent correlation between isolation from the vagina and inflammatory lesions has not been identified. *H. somnus* should be considered as a potential cause of infertility when a heavy growth is isolated from animals with post-breeding endometritis, vaginitis, and purulent vaginal discharge. Care should be taken to rule out other causes of infertility including trichomoniasis, ureaplasmosis, venereal campylobacteriosis, and infectious pustular vulvovaginitis.

Abortion. Diagnosis is based on recovery of the organism from the placenta or fetus. Isolation of a heavy and pure growth of the organism from fetal tissues generally is diagnostic. Isolation of a light growth from the placenta could be due to contamination in the birth canal,

however, and culture results should be interpreted in conjunction with clinical confirmation of placental lesions, particularly vasculitis.

Treatment and Control

Infertility. Antibiotic treatment has been reported to increase fertility in herds affected with *H. somnus*–induced vulvovaginitis. *H. somnus* is sensitive to most antibiotics at therapeutic levels, including second- and third-generation penicillins, florfenicol, tetracycline, and sulfas. Treatment with florfenicol or ceftiofur has lead to good clinical response. Vaccination also has been reported to result in a return to a normal reproductive schedule within several months.

Abortion. Because *H. somnus* reaches the conceptus by hematogenous dissemination, vaccination probably would be of value in preventing abortion due to this agent. Controlled studies to determine the efficacy of vaccination for the prevention of abortion have not been undertaken, however. Because *H. somnus* typically causes sporadic abortions, vaccination to specifically prevent abortion is rarely warranted.

Leptospirosis

Leptospira abortion in cattle is not a single disease with common risk factors, epidemiology, host response, or means of control. It can be separated into relatively distinct syndromes depending on whether disease is caused by host-adapted strains or whether the cow is an incidental or accidental host. Cattle are a maintenance host for hardjo. A maintenance host relationship is characterized by efficient transmission between animals, a relatively high incidence of infection, production of chronic rather than acute disease, and persistence of infection in the kidney and genital tract (BP). Diagnosis of infection in maintenance hosts often is difficult because of a relatively low antibody response and the presence of few organisms in tissues. Infection of incidental or accidental hosts leads to acute disease, sporadic transmission within the host species, and a short phase of renal infection. Incidental infections are typified by a marked antibody response, with large numbers of organisms in the tissues of infected animals.

Agent, Source, and Epidemiology

Organisms of the genus *Leptospira* are small aerobic spirochetes that can be found in a wide variety of animal species (parasitic strains) and in water (saprophytic strains). Pathogenic leptospires were formerly classified as members of the species *L. interrogans*. The genus has recently been reorganized, and pathogenic leptospires are now identified in seven different species. Greater than 200 different serovars have been identified throughout the world.

Each serovar tends to be maintained in specific maintenance hosts. Hardjo is the serovar maintained in cattle. Two major genotypes of hardjo are found in cattle: **hardjobovis** and **hardjoprajitano.** Serovar pomona is maintained in swine and a variety of free-living animals and is the most important source of incidental infection of cattle in the United States, Australia, and New Zealand.

Transmission among maintenance hosts often involves direct contact with infected urine, placental fluids, or milk. Transplacental and venereal transmission also can occur. Infection of incidental hosts typically is acquired by indirect means, such as contact with water sources or an environment contaminated by the urine of maintenance hosts. The relative importance of incidental infections is dependent on management and environmental factors, which determine the opportunity for contact and transmission of the organism from other species to cattle. Warm, moist conditions with a pH near neutral favor the survival of leptospires. Because of this, infection by incidental leptospires is most common in spring, summer, or fall in temperate regions. Environments favorable to the survival of leptospires are much less important in the epidemiology of host-maintained species.

Pathogenesis

Infection of susceptible animals occurs through the mucous membranes of the eyes, nose, vagina, and penis and through abraded or water-softened skin. A bacteremic phase follows after a 4- to 10-day incubation period. This phase typically is subclinical, but bacteremia may be associated with acute clinical disease. After leptospiremia develops, the organism localizes to and persists in a number of organs, including the kidney and the genital tract of males and females. Leptospires are shed in urine and in the postcalving uterine discharge. They also may localize in the testes and accessory glands and can be demonstrated in bull semen. Venereal transmission of hardjo in cattle is common.

Leptospires localize and multiply in the proximal tubules and are voided in the urine. With hardjo infection in cattle, the organism is shed consistently in urine for 4 to 6 weeks and intermittently for 6 to 12 months. Shedding in urine may persist for life. Urinary shedding with hardjoprajitano is of very low intensity, and the venereal route probably is more important. Localization and persistence of the organism in the uterus may result in fetal infection with subsequent abortion, stillbirth, or birth of a weak calf.

Clinical Signs

The vast majority of leptospiral infections in nonpregnant cattle are subclinical. Fetal infection will result in abortions, stillbirths, and the birth of weak calves. Hardjo and pomona are the most commonly implicated serovars. Abortion occurs 1 to 6 weeks (pomona) to 4 to 12 weeks (hardjo) after the acute phase of infection. Pomona abortions generally occur in the last trimester. Hardjo may cause infertility, or abortion from 4 months of gestation to term. The incidence of abortion on individual farms can be very high (up to 50%) after an epizootic of pomona infection. Abortion rates tend to be much lower with hardjo (3%–10%) but occasionally may approach 30%. Infertility has been a common field observation in hardjo-infected herds. Infertility apparently responds to vaccination and treatment.

Pathology

Consistent gross lesions are not identified. Occasional fetuses appear jaundiced. Microscopic lesions may be observed in the kidney and consist of foci of tubular necrosis with interstitial and perivascular infiltrates of lymphocytes and a few plasma cells. Renal inflammation is not specific for leptospirosis because other agents, including *Neospora,* may cause similar lesions. Bile plugs in canaliculi may be identified in the liver.

Diagnosis

Diagnosis of *Leptospira* abortion is based on demonstration of leptospires in fetal tissues or the identification of a serologic response in the dam or the fetus. Because isolation and identification of leptospires constitute a specialized, difficult, and time-consuming process, it is rarely undertaken as a routine diagnostic procedure. Kidney generally is the tissue of choice for diagnosing *Leptospira* infection in the fetus, and the organism can be identified by a direct FA test on fresh, minimally autolyzed kidney or with IHC studies on fixed kidney. Postmortem autolysis can limit the usefulness of both IHC and FA tests. PCR assay appears to be the most sensitive procedure to diagnose leptospiral abortion but is not widely available.

The fetus is able to mount a serologic response by 4 to 5 months of gestation. Because maternal antibody does not cross the placental barrier, the presence of leptospiral antibodies in fetal thoracic fluid is indicative of in utero infection.

Interpretation of results of *Leptospira* serologic studies in the dam is complicated by a number of factors, including cross-reactivity of antibodies, vaccination titers, and differences in response to host-adapted versus non–host-adapted serovars. Paired serum samples from aborting cows typically are of no value, because titers are either maximal (for non–host-adapted strains), falling, or static at the time of abortion (for host-adapted strains). A finding of high titer (≥1 : 1000) at the time of abortion in an individual animal that has not been recently vaccinated suggests a strong possibility of *Leptospira* abortion; high titers are common after abortion due to non–host-adapted strains. Unfortunately, the converse is not true. Approximately one third of cows that aborted *Leptospira*-infected fetuses had low titers (<1 : 100). Often, at the time of abortion, antibody titers are quite low, or antibody may even be undetectable, in maintenance hosts. Maintenance hosts may be actively infected and shedding leptospires with antibody titers of 100 or less. For chronic hardjo infections, the chance of fetal infection is approximately 60% in a recently aborting cow with a titer of 300 or greater, 80% with a titer of 1000 or greater, and 90% with a titer of 3000 or greater. A herd test approach is not very useful for the diagnosis of hardjo-associated abortion in endemically infected herds, because low titers are common after abortion. The presence of bleeding in 10 animals, or 10% of the herd, with the identification of titers to hardjo of 300 or greater indicates active infection but is not necessarily diagnostic for *Leptospira* abortion.

Treatment and Control

Control of both incidental and host-maintained leptospirosis is based on vaccination and limiting exposure. To limit exposure to non–host-adapted leptospires, it is desirable to reduce contact with wildlife, control rodents, and eliminate access to potentially contaminated water sources (such as by fencing off sloughs, ponds, and streams). Vaccination against incidental serovars generally provides good protection against challenge.

Control of host-adapted strains is more difficult. In outbreaks, antibiotics can be used to try to diminish exposure by decreasing or eliminating shedding. A variety of antibiotics, including streptomycin, tetracycline, erythromycin, tiamulin, and tylosin, have been reported to be effective against leptospires. The most effective antibiotic, streptomycin, can no longer be used in cattle in the United States, however. Antibiotics may diminish shedding but have been disappointing as a means of eliminating infection.

Annual vaccination with a polyvalent product typically is the foundation of a *Leptospira* control program. The first vaccination should be administered after the age of 6 months. Animals should initially be vaccinated twice, followed by annual vaccination. In problem herds, twice-yearly vaccination may be necessary because of the often short-duration, low-titer immunity. Because the predominant vaccine strain of hardjo is hardjoprajitano, vaccines may not provide good protection against hardjobovis.

Listeria Infection

The principal diseases caused by *Listeria monocytogenes* in ruminants include encephalitis, abortion, and neonatal septicemia. It is the exception for different conditions to occur in the same flock or herd. *Listeria* is widely distributed in nature, having been isolated from soil, vegetation, decaying vegetation, water, feces, sewage sludge, and tissues from a wide variety of vertebrate and invertebrate species. Especially in temperate regions, exposure to *L. monocytogenes* is common, although disease is not.

In pregnant animals, *Listeria* has a predilection for fetoplacental tissues. Fetal infection results in abortion, generally during the last trimester. Because of the ubiquitous nature of the organism, *Listeria* may cause sporadic, individual animal abortions. Outbreaks of *Listeria* abortion are more common in the winter and generally are the result of ingestion of *Listeria*-contaminated feed, particularly improperly cured silage. Although abortion rates of up to 50% have been reported, the abortion rate rarely exceeds 15%.

Agent, Source, and Epidemiology

L. monocytogenes is a gram-positive coccobacillus. The genus *Listeria* contains five species, of which *L. monocytogenes* is the principal pathogen. Bovine abortions are most commonly caused by serovars 1 and 4b. *Listeria ivanovii* occasionally has been associated with bovine abortion.

The natural habitat of *L. monocytogenes* appears to be the environment, and the organism is widespread. Ingestion of *L. monocytogenes* results in an animal-environmental cycle, and during an outbreak of listeriosis, the bacterium can be isolated from the feces of a large

percentage of healthy animals. The aborted fetus, placenta, and uterine discharge contain myriad *Listeria* organisms, and contamination of food or water sources with this material may be a source of infection for other animals.

Most outbreaks of listeriosis have been traced to feeding pregnant animals spoiled feeds containing massive numbers of *Listeria* organisms, most commonly improperly cured or spoiled silage. The bacteria can proliferate in rotting vegetation, in which aerobic conditions and a pH higher than 5.4 are more likely. Additional sources of *Listeria* include decayed forage at the bottom or corners of feed bunks and rotted hay at the periphery of hay bales or haystacks. The incidence of disease due to *Listeria* is increasing, possibly as a result of changes in the handling of bulk feedstuffs, which may lead to a greater degree of spoilage.

Pathogenesis

L. monocytogenes is capable of entering cells of the monocyte-macrophage series, escaping from the phagosome, multiplying within the cytoplasm, and spreading between cells. Crucial to the virulence of *L. monocytogenes* is its ability to escape intracellular killing within macrophages by lysis of the phagosomal membrane and move into the cytoplasm, which is mediated by the secretion of a hemolysin, listeriolysin.

Oral infection does not consistently produce abortion. Abortion is readily produced by the intravenous inoculation of pregnant ruminants. Fetal infection is considered to be the result of hematogenous spread from the placenta. The incubation period from infection to abortion is typically 5 to 12 days.

Clinical Signs

A majority of *Listeria* abortions occur during the last trimester, although abortion may occur as early as the fourth month of gestation. *Listeria* abortions differ from many other bacterial abortions in that clinical signs often are present in the cow before, during, and after abortion. These include weight loss, fever, an inflammatory leukogram, endometritis, and retained fetal membranes. The effect on fertility usually is transient, and aborting animals tend to resist reinfection. In herds experiencing *Listeria* abortions, encephalitis seldom occurs in conjunction with reproductive disease.

Pathology

Affected fetuses often are retained in utero for several days before expulsion and typically are autolyzed. Abortion is the result of an acute placentitis with subsequent fetal septicemia. Although often difficult to visualize owing to autolysis, placental lesions may consist of pinpoint, yellowish foci involving the tips of cotyledonary villi with focal to diffuse intercotyledonary placentitis. The fetus usually is autolyzed, and miliary foci of necrosis may be seen in the liver or spleen. These foci may be confused with necrotizing lesions associated with IBR abortion.

Diagnosis

Diagnosis is based on isolation of the organism from fetal tissues or placenta. Unlike in *Listeria* encephalitis, in which culturing the organism from brain may be difficult, *Listeria* is readily recovered from fetal tissues and placenta—the fetus typically dies as a result of a septicemia, so that bacteria are present in high numbers in all tissues and fluids, placenta, and the uterine discharge. Microscopic lesions may consist of an acute placentitis and necrotizing to necrosuppurative hepatitis. Bacterial colonies often are observed in the lumen of vessels throughout the fetus and in the center of hepatic lesions.

Gram staining of fetal fluids or tissue impression smears will reveal numerous gram-positive, pleomorphic coccobacilli. A polyvalent (serotypes 1 to 4) FA conjugate is available for the identification of organisms in impression smears. Presence of *Listeria* in fixed tissues also can be confirmed by IHC studies.

The importance of *L. monocytogenes* as a food-borne pathogen had earlier prompted the development of reliable and highly discriminating typing methods for epidemiologic purposes. These include DNA fingerprinting and pulsed-field gel electrophoresis. The use of molecular methods has enabled the discrimination of individual strains of *Listeria,* which could aid in investigating potential sources of the organism in epizootics.

Treatment and Control

The connection between *L. monocytogenes* and silage feeding is well established. The wide distribution of the organism in nature makes it unlikely that silage will be totally free of *Listeria*. Therefore, an emphasis should be put on proper curing of silage and elimination of the feeding of spoiled material to pregnant animals.

Because *Listeria* is an intracellular parasite, killed vaccines have not been effective. Modified live vaccines have shown promise in other species but at present are not available for use in cattle. If potentially contaminated material must be fed to pregnant animals, therapeutic doses of antibiotics, such as tetracycline, can be added to the feed or water. Antibiotic therapy is contraindicated in dairy herds because of the problem of residues in the milk.

Mycoplasma and *Ureaplasma* Infection

Considerable field and experimental evidence is available to support the ability of *Ureaplasma diversum* and *Mycoplasma* spp. to cause infertility and abortion in cattle. Unfortunately, a diagnosis of infertility due to these agents is complicated by the fact that they may be normal inhabitants of the urogenital tract of cows and bulls and frequently are isolated in the absence of disease.

Field studies have linked *Ureaplasma diversum* to vulvitis, infertility, and abortion, and all three syndromes have been experimentally reproduced. Apparent outbreaks of *Ureaplasma*-induced infertility have been reported, with conception rates as low as 20%. Reproductive problems may persist for up to 6 months. *Ureaplasma* also has been associated with bovine abortion. Unfortunately, few diagnostic laboratories routinely attempt to isolate *Ureaplasma* from aborted fetuses, so current understanding of the importance of this agent as a cause of abortion is based largely on information from a single laboratory. Over a 7-year period beginning in

1994, *Ureaplasma* abortion was diagnosed in 2.3% (range 0.9–3.9%) of aborted bovines at this laboratory. *Ureaplasma* was the second most common bacterial cause of abortion reported during this period, trailing only *A. pyogenes* (3.0%). The incidence of *Ureaplasma* abortion has been reported to be as high as 10%.

Mycoplasma bovigenitalium has been linked to granular vulvovaginitis, endometritis, and infertility in the cow and seminal vesiculitis in the bull. *M. bovis* infects the reproductive tract of bulls less commonly than *M. bovigenitalium*. *M. bovis* may represent a more important danger after venereal transmission, however, because of its proven pathogenicity for the cow's reproductive tract. Endometritis, salpingitis, infertility, and abortion have been produced by *M. bovis* in experimentally infected cows. Seminal vesiculitis, epididymitis, and orchitis due to *Mycoplasma* infection have been reported in bulls.

A variety of mycoplasmas have been isolated from aborted bovine fetuses. Only *M. bovis* (and *Mycoplasma mycoides*) has consistently caused abortion when inoculated into pregnant cows. *Mycoplasma* spp. appear to be an uncommon cause of abortion in cattle. In one large prospective survey, *Mycoplasma* organisms were not isolated from any of the 794 fetal samples evaluated. Reports typically associate *Mycoplasma* infection with sporadic abortions.

Agent, Source, and Epidemiology

Both *Ureaplasma diversum* and *Mycoplasma* spp. belong to the family Mycoplasmataceae and are small (0.3–0.8 μm in diameter), cell wall–deficient, pleomorphic organisms. *M. bovigenitalium* and *U. diversum* are commonly carried in the vulva and vagina of females and in the distal urethra, prepuce, and semen of males. *Ureaplasma* also is commonly carried in the nasal passages. These organisms are readily transmitted by natural breeding and contaminated insemination. Additional modes of transmission include direct contact, infection during passage through the birth canal, and environmental contamination by urine from infected animals.

Pathogenesis

Salpingitis commonly is observed in cattle with *Mycoplasma* or *Ureaplasma* infection. The induction of salpingitis, cervicitis, and endometritis after natural service or artificial insemination is the likely mechanism of infertility.

Clinical Signs

In the cow, clinical signs associated with *Ureaplasma* infection include granular vulvitis, infertility, and abortion. In utero *Ureaplasma* infection also has lead to the birth of weak calves. Direct inoculation with *Ureaplasma* organisms has produced seminal vesiculitis in bulls, and an association with posthitis has been reported. *Mycoplasma* infection of the reproductive tract may result in granular vulvovaginitis, infertility, and abortion.

Pathology

In cases of *Ureaplasma* abortion, the intercotyledonary chorioallantois is thickened, opaque, and white to brown, and the cotyledons may be cupped. Histologic examination reveals placentitis characterized by a mononuclear inflammatory cell infiltrate accompanied by fibrosis, necrosis, and mineralization with associated vasculitis of chorioallantoic and amniotic vessels. Nonsuppurative alveolitis with hyperplasia of bronchiolar-associated lymphoid tissue also is generally apparent.

Placentitis, suppurative fetal bronchopneumonia, and subacute myocarditis and epicarditis have been reported in cases of *Mycoplasma* abortion.

Diagnosis

Infertility. The first step in establishing a diagnosis of infertility due to *Mycoplasma* or *Ureaplasma* is isolation of the organism. Unfortunately, this initial step is fraught with a number of pitfalls. These organisms are fastidious, and the special procedures and expertise required for their isolation are not available at all laboratories. Because of the fragile nature of both organisms, proper collection techniques and preservation are essential. To isolate either *Mycoplasma* or *Ureaplasma* organisms, Dacron- or polyester-tipped swabs should be used to collect vulvar and vaginal samples. Areas of active vulvar inflammation tend to be the most productive. Swabs should be immediately submersed in transport medium (without charcoal) and frozen. Samples must remain frozen until arrival at a laboratory. PCR assay also has been used to detect *Ureaplasma* or *Mycoplasma* organisms but typically is not available for routine diagnostic procedures. Caution must be exercised when either *U. diversum* or *M. bovigenitalium* is under consideration as a cause of infertility, because both are commonly isolated from healthy animals, and concurrent evaluation for other causes of infertility is warranted.

Abortion. Preferred specimens for the isolation of *U. diversum* or *Mycoplasma* spp. include lung, caruncle, cotyledon, stomach contents, and amniotic fluid. These samples should be immediately refrigerated and presented to a laboratory the same day or immediately frozen and shipped frozen. Isolation of either organism from the conceptus is significant only in conjunction with clinical confirmation of characteristic lesions.

Treatment and Control

Artificial insemination may play a role in *Ureaplasma*-induced infertility. *Ureaplasma* can be carried into the uterus during artificial insemination; subsequent infection of the upper reproductive tract may lead to infertility. Use of a guarded insemination instrument is of value in preventing uterine contamination. Tetracycline (1 g), administered by intrauterine infusion 1 day after insemination, has been used to improve fertility in herds with confirmed *Ureaplasma*-related reproductive problems. Tylosin (10 mg/kg) for 5 days was reported to be an effective treatment for *Ureaplasma*-induced vulvitis and posthitis.

Fluoroquinolone antibiotics, such as enrofloxacin, have shown promise against mycoplasmas and may be useful for the treatment of reproductive disease caused by *Mycoplasma* spp. Tylosin and tetracycline also typically demonstrate good activity against *Mycoplasma* spp.

Chlamydia Infection

In cattle, chlamydial infection has been associated with keratoconjunctivitis, pneumonia, enteritis, polyarthritis-polyserositis, encephalomyelitis, mastitis, seminal vesiculitis, infertility, and abortion. Unlike in sheep and goats, in which this organism is one of the most important causes of abortion, the paucity of reports in cattle suggests that *Chlamydia* is an uncommon pathogen of the bovine reproductive tract. In one study in which more than 250 fetuses were evaluated, *Chlamydia* was not identified in a single aborted bovine.

Agent, Source, and Epidemiology

Chlamydiae are obligate, intracellular parasites that multiply within the cytoplasm of eukaryotic cells. The life cycle has two distinct forms: **elementary bodies** (200–300 nm), which are involved in attachment and penetration of susceptible host cells, and **reticulate bodies** (500–1000 nm), which are the replicating form. Reticulate bodies mature into a new generation of infectious elementary bodies, which are then released by cell lysis. Strains of *Chlamydia* associated with genital tract infections in the bovine typically are immunotype 1.

In ruminants, the intestinal epithelium appears to be an important natural habitat for *Chlamydia*. Persistent intestinal infections are common in cattle, sheep, and goats. The organism may be shed in feces, nasal, ocular, or vulvar discharges or in uterine fluids, placenta, or urine. Infection may be established by ingestion or inhalation of these materials.

Chlamydia has been identified in the testes, epididymis, accessory sex glands, kidney, semen, and urine of experimentally inoculated bulls. Infertility and repeat breeding were reported in cows bred by bulls naturally shedding chlamydiae in semen.

Pathogenesis

Infertility. *Chlamydia* does not appear to interfere with fertilization, nor does it penetrate the zona pellucida. The agent multiplies within endometrial cells, leading to endometritis. Uterine inflammation is the likely mechanism of early embryonic death.

Abortion. The placental junction is breached during chlamydemia, which results in placental and fetal infection. Cows inoculated intravenously with *Chlamydia* organisms aborted within 5 to 38 days, whereas pregnant cows inoculated by the intramuscular, subcutaneous, or intradermal route aborted or gave birth to weak calves 33 to 126 days later.

Clinical Signs

Chlamydia infection has been associated with infertility, repeat breeding, and abortion. Chlamydial abortions typically are sporadic, although in some herds as many as 20% of pregnant cows may abort. Abortion has been detected as early as the fifth month of gestation, but most occur during the last trimester. The birth of weak calves also has been reported in association with *Chlamydia* infection. Retained placentas have been observed after both experimental and natural abortions.

Pathology

The hallmark of chlamydial abortion is a placentitis, which is manifested as thickening and necrosis of the intercotyledonary placenta with necrosis of cotyledonary villi. A necrotizing vasculitis with edema often is observed in the placenta. Late-gestation fetuses also may exhibit abdominal distention due to ascites; an enlarged, swollen, mottled red-yellow liver; and diffuse lymph node enlargement.

Diagnosis

A diagnosis of chlamydial abortion relies on demonstration of the organism in fetal tissues or placenta, the detection of antibodies in fetal thoracic fluid, or the demonstration of a serologic response in the dam. Sample selection is of primary importance for the diagnosis of chlamydial infection. Preferred samples include placenta or the discharge from a recently aborting cow. *Chlamydia* is less consistently identified in fetal liver or lung. A tentative diagnosis can be based on the identification of small, intracytoplasmic organisms in Gimenez-stained placental impression smears. A definitive diagnosis is based on positive identification of *Chlamydia* with FAs on placental impression smears, or detection of the agent in uterine discharge, placenta, fetal lung, or liver by culture, antigen-capture ELISA, or PCR assay. These procedures also can be used to detect chlamydiae in semen or uterine discharges in cases of infertility. IHC studies have been used to identify chlamydiae in fixed placenta or liver.

Delivery of a *Chlamydia*-infected fetus stimulates a rapid rise in antibody titer that reaches maximum levels 14 to 21 days after the termination of pregnancy. Accordingly, paired serum samples obtained at the time of abortion and 2 to 3 weeks later should have a significant rise in titer. Because chlamydial infection typically causes late-gestation abortion after chronic infection of the fetus, seroconversion often can be demonstrated in fetal fluids and is diagnostic for chlamydial abortion.

Treatment and Control

Vaccines have been used to prevent chlamydial abortion in other species. An approved vaccine is not available for use in cattle, however. Use of vaccines intended for other species may not be effective owing to antigenic differences between strains. Chlamydiae are susceptible to tetracycline, and this antibiotic has been used both as a preventive and for treatment in other species. Because premonitory signs are absent and *Chlamydia* infection typically is associated with only sporadic abortions in cattle, however, the use of tetracycline for prevention generally is not warranted.

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CHAPTER 50

Viral Diseases of the Fetus

CLAYTON L. KELLING

BOVINE VIRAL DIARRHEA VIRUS INFECTIONS

Bovine viral diarrhea virus (BVDV) is one of the most commonly encountered and economically important pathogens of cattle in North America. Since the mid-20th century, BVDV has been recognized as a significant cause of disease of the gastrointestinal system. The impact of BVDV on reproduction was not perceived for another 30 years, when the occurrence of persistent infection in immunotolerant cattle was described.

BVDV infections may occur in cattle as acute illness—that is, bovine viral diarrhea (BVD)—or as a generally chronic condition—mucosal disease. When susceptible pregnant cattle are infected with BVDV, transplacental infections usually occur. Transplacental infections may lead to embryonic or fetal death and abortion, to developmental defects of organs, or to development of immunotolerance and establishment of persistent infections. Acute BVDV infections contribute, through immunosuppression, to causing multifactorial diseases, such as diseases of the respiratory and enteric tracts in susceptible calves.

Clinical Forms of Infection with Bovine Viral Diarrhea Virus

The clinical form of BVDV infection—inapparent or severe BVD, reproductive failure, persistent infection, or mucosal disease—observed within a herd is dependent on interaction of several factors at the time of infection. These determining factors include the biologic properties of the virus, the age and stage of gestation of pregnant cattle, level of immunity of the herd, and the interplay of stressors.[1]

Acute Infections

BVD is an acute postnatal infection in seronegative, immunocompetent cattle. The clinical severity of acute BVDV infections is variable, but a majority of postnatal BVDV infections are inapparent. Milder forms of BVD are characterized by high morbidity, low mortality, a normal host immune response, and minimal mucosal lesions. Usual findings include pyrexia, nasal discharge, and transient leukopenia. Viremia lasts for 3 to 10 days (acute infections with higher virulence isolates may result in viremia of longer duration) and antibody titers rise slowly

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for 3 months after infection.[2] Severe acute BVD outbreaks with marked thrombocytopenia, hemorrhages, and high mortality rates have been associated with infection with high-virulence BVDV isolates.

Acute BVDV infections contribute to causing multifactorial diseases through immunosuppression. Immunosuppression is mediated by suppression of immune functions through the lymphotropism of BVDV. BVDV lymphotropism results in depletion of lymphocytes from lymphoid tissues. Immunosuppression due to BVDV infection enhances the severity of bovine rotaviral enteritis in calves, in addition to directly causing enteritis.[3] BVDV-induced immunosuppression predisposes calves to development of naturally occurring bovine respiratory tract disease (BRD). Indirect effects of BVDV in causing BRD were demonstrated in experimental bovine respiratory syncytial virus (BRSV) and BVDV co-infections in which more severe respiratory tract and enteric disease occurred than in infections with either virus alone.[4] Subpopulations of lymphocytes were more markedly altered in peripheral blood and lymphoid tissues from co-infected calves than in calves infected with either BRSV or BVDV alone. Co-infected calves had a reduction in the percentage of T lymphocytes (including $CD8^+$ lymphocytes and $CD4^+$ lymphocytes) in the thymus and Peyer's patches.[5] An additional finding in these calves was more extensive pneumonia, characterized by caudodorsal as well as cranioventral interlobular edema, emphysema, and bronchopneumonia in caudal lung lobes. By contrast, calves infected with BRSV alone had only cranioventral bronchopneumonia.

Transplacental Infection

Transplacental infection is likely to occur in susceptible, pregnant cattle infected with BVDV. The outcome of transplacental infection is dependent on the biologic properties of the infecting virus, especially the biotype of the virus, and the stage of gestation at the time of infection. The potential outcomes of transplacental infections—embryonic or fetal death and abortion, developmental defects of organs, and development of immunotolerance with establishment of persistent infections—are discussed later in the chapter.

Persistent Infection

Fetal infection with noncytopathic BVDV can result in the birth of calves with persistent BVDV infection. The primary means of producing a persistently infected calf is through transplacental infection after a primary acute infection in a pregnant cow, although persistently infected cows (i.e., congenitally infected) also will give birth to persistently infected calves. Persistently infected animals shed large amounts of virus and are therefore carriers and a primary source of exposure for susceptible cattle.[6] In most instances they do not produce detectable antibodies to BVDV, because they are immunotolerant to the virus. Some calves with persistent infection are stunted or weak at birth, have poor growth rates, and die at a young age.[7] Others appear healthy and survive to maturity. The prevalence of cattle with persistent infection is variable; however, on the basis of sampling of randomly selected herds, it has been estimated that 4% of herds in the United States have persistently infected calves.[8] Such animals are at risk of developing mucosal disease.

Mucosal Disease

Mucosal disease, associated with high mortality rates, occurs sporadically (low morbidity) in cattle that usually are between 6 months and 2 years old but may be of any age. Characteristic clinical manifestations include anorexia, pyrexia, diarrhea, loss of condition, and death.[2] Gross pathologic lesions may include erosive or ulcerative lesions on the muzzle and lips, buccal mucosa, and tongue. Commonly, elongated ulcerative lesions occur in the mucosa of the esophagus. Erosions also may be found on the rumen pillars, reticulum, and abomasum. Enteritis may be evident and may vary in presentation from catarrhal to hemorrhagic to erosive/ulcerative. Peyer's patches and lymphoid tissue in the proximal colon may be hemorrhagic.[9] Thymus atrophy and enlarged peripheral lymph nodes are prominent features.

Biologic Properties of Bovine Viral Diarrhea Virus

BVDV is a member of the genus *Pestivirus,* family Flaviviridae,[10] which also includes border disease virus of sheep and hog cholera virus. Pestiviruses are small, enveloped, single-stranded, positive-sense RNA viruses that are antigenically related.

The host range for BVDV comprises domestic or wild ruminants and swine. Pestiviruses are presumed to persist in the environment for no more than two weeks, and are readily inactivated by common disinfectants. Therefore, virus transmission is primarily vertical or by inhalation or ingestion of material contaminated with infected body secretions and excretions (saliva, oculonasal discharge, urine, feces, semen, uterine secretions, placenta, and amniotic fluid) of infected animals.

Isolates of BVDV vary in their relative virulence potentials, which accounts in part for variability in severity of lesions and clinical disease among different cases.[11] BVDV isolates are divided into two biotypes (groups of viruses with the same genetic composition) based on their ability to induce microscopically visible changes (vacuolization and lysis) in host cells in vitro: cytopathic and noncytopathic. BVDV strains are divided into two genetic groups or genotypes—1 and 2—using gene sequencing techniques and cross-neutralization assays. RNA viruses, including BVDV, are prone to mutate; therefore, BVDV has high potential to mutate in response to selective immune pressure. Mutation is the putative strategy used by BVDV to escape the host's immune response and to persist in the cattle population. Antigenic diversity among field isolates has important implications for development of protective immunity.

Biotypes: Noncytopathic and Cytopathic

The two biotypes of BVDV, cytopathic and noncytopathic, have separate biologic roles[2]; biotype differences are important in disease pathogenesis. Both biotypes of BVDV infect cattle and cause disease, but only the noncytopathic isolates cause persistent infections. Isolates

that have the ability to cause microscopically visible changes in host cells (vacuolization and lysis) are assigned to the cytopathic biotype. Isolates lacking this capability are assigned to the noncytopathic biotype. Cells infected with cytopathic BVDV have an 80-kilodalton (kD) polypeptide that is distinguishable electrophoretically from cells infected with noncytopathic viruses, which do not have the polypeptide.[12] This 80-kD nonstructural viral protein apparently plays a crucial role in replication of cytopathic viruses. Diversity in antigenicity among strains is not discretely separable. No link exists between biotype and antigenicity, and strains that are antigenically distinct overlap both biotypes, so protective immunity afforded by a vaccine is not dependent on the biotype of the vaccine virus.

In the laboratory, the presence of noncytopathic BVDV constitutes a significant quality control issue for workers in diagnostic laboratories as well as for manufacturers of vaccines. This is because noncytopathic BVDV isolates commonly occur in commercial fetal calf sera used to supplement cell culture media used in cell cultures to grow viruses. In the diagnostic laboratory, when noncytopathic BVDV occurs undetected as a contaminant of cell culture, accuracy of diagnostic laboratory assays, such as virus isolation tests and serum neutralization tests, is compromised. Noncytopathic BVDV contamination of modified live virus vaccines during the manufacturing process has represented a significant risk factor since these products were introduced.[13] The potential for contamination of cell cultures with noncytopathic BVDV is a continual concern, because between 20% and 50% of commercial fetal bovine serum lots are virus positive[14] for both genotypes.[15] Fetal bovine serum quality assurance procedures applied before use in diagnostic laboratory testing or in cell culture production systems to grow vaccine virus include rigorous virus testing followed by the additional precautionary measure of irradiation or chemical treatment.[14]

Genotypes

BVDV strains are divided into two genetic groups or genotypes using gene sequencing techniques and cross-neutralization assays.[16,17] Genotype 1 isolates are primarily classic laboratory reference and vaccine strains. Genotype 2 viruses are found predominantly in fetal bovine serum, persistently infected calves born to dams vaccinated against BVDV, and the more recently described BVDV strains associated with high mortality and acute and peracute infections involving hemorrhage. Biotype, genotype, and antigenic cross-reactivity vary independently,[18] as do biotype, genotype, and pathogenicity.[19] The antigenic differences between genotype 1 and genotype 2 isolates and the clinical importance of genotype 2 BVDV isolates constitute the basis for the recognition that to be effective, vaccines must provide broad cross-protective immunity against both genotype 1 and 2 isolates.

Outcomes of Fetal Infections with Bovine Viral Diarrhea Virus

BVDV enters the susceptible host primarily by the oronasal route and replicates in tonsils, lymphoid tissues, and epithelium of the oropharynx. Phagocytic cells take up BVDV or virus-infected cells, or both, for transport to lymphoid tissues.[20] Viremia is evident 2 to 4 days after exposure. Viremia in a pregnant female is certain to lead to transplacental infection and fetal infection. The outcome of fetal infections with BVDV is determined primarily by the stage of fetal developmental at the time of infection, and by biotype and virulence of the infecting virus.[21] The stage of development of the evolving fetal immune system at the time of infection plays a major role in determining the outcome of infections.[21] Transplacental infections are particularly damaging during the first two trimesters of gestation and may result in persistent infections, fetal death and abortion, or congenital developmental defects.[22] Persistent infection in calves is the most significant outcome of fetal infection because of the negative effects such infection has on herd production. Persistently infected calves are the most important source of virus to perpetuate disease within and between herds. Moreover, persistently infected calves usually have poor growth rates and die at a young age. Reproductive failure mediated by abortion and birth of calves with congenital abnormalities also are significant outcomes of fetal BVDV infections that adversely affect herd performance.

Persistent Infections

Persistent BVDV infections may be established if infection of the fetus occurs during the third or fourth month of gestation before immunocompetence becomes established.[21,23] Viremia of the pregnant dam, stemming from either a persistent or an acute infection, is the source of the virus that infects the fetus. Before infecting the fetus, BVDV replicates in the placenta. Persistent viremia develops as a result of fetal immunotolerance and failure to develop antibodies against the persisting virus.[6] Persistently infected calves are carriers because they are viremic and shed virus continuously, and they may spread virus within and between herds. The level of viremia may decline with the development of neutralizing antibody and become undetectable as the animal ages[24] as a result of deterioration of highly specific immunotolerance to the persisting virus.[25] Deterioration of immunotolerance, eventuating in an immune response, may result from development of antigenic-variant viruses within the immunotolerant, persistently infected animal.[25] Persistently infected calves frequently are "poor doers," have reduced growth rates, are more susceptible to common calfhood infections of mucosal surfaces including pneumonia and enteritis, and are at risk of developing mucosal disease.[26,27]

Abortion

BVDV infections of fetuses during the first and second trimesters may cause fetal death and abortion. Third-trimester abortions also have been attributed to BVDV infection.[21] Individual isolates probably vary in their ability to cause abortion. The rate of abortion under field conditions is variable, but abortion rates as high as 40% have been reported after experimental infections on day 100 of gestation.[22]

Congenital Defects

Congenital defects may result if infection occurs during midgestation (100–150 days). Congenital defects associated with BVDV infections may involve the nervous system (microencephaly, cerebellar hypoplasia, hydranencephaly, hydrocephalus, and hypomyelination), eye (cataracts, retinal degeneration, optic neuritis, and microphthalmia), immune system (thymic aplasia), integumentary system (alopecia and hypotrichosis), musculoskeletal system (brachygnathism, growth retardation, and arthrogryposis), or respiratory system (pulmonary hypoplasia).[28] The pathogenetic mechanisms for development of defects are not known. Because fetal organs and immune system (inflammatory response) are developing during this stage, direct cell damage by viral infection and destruction of virus-infected cells by the evolving immune system are possible mechanisms.[21]

Late-Gestation Transplacental Infections

The outcome of BVDV infections during late gestation (last trimester) is comparable with that with acute postnatal infections of cattle. At this time the fetal immune system has developed to respond efficiently against BVDV infection. Consequently, transplacental infections during late gestation are not associated with a significant level of congenital defects. Third-trimester abortions have been attributed to BVDV infection.[21] The most common outcome of infections during this period is birth of a clinically normal calf with high levels of precolostral antibodies.[21,22]

Diagnosis of Bovine Viral Diarrhea Virus Fetal Infections

Identification of Persistently Infected Cattle

Persistently infected carrier cattle are identified in herds on the basis of tests conducted in a diagnostic laboratory. The tests include (1) the virus isolation (VI) test, (2) the immunohistochemistry (IHC) test, (3) the polymerase chain reaction (PCR) assay, and (4) the enzyme-linked immunosorbent assay (ELISA).

Virus isolation test. The standard VI test format (macrotest), a highly reliable test,[7,29] is not practical for testing a large herd. The standard VI test may be used to test mononuclear cell preparations (buffy coats) from blood samples collected in tubes with anticoagulants. The cells are washed to limit interference from antibodies, which reduce test sensitivity. An adaptation of the standard VI test is the immunoperoxidase microtiter plate VI assay, which is relatively sensitive and specific and is designed to efficiently test large numbers of serum samples, such as in herd testing programs.[30,31] Blood is collected for virus isolation from calves that are 2 months of age or older, when maternal antibody titers have declined, because maternal antibodies reduce the ability to isolate BVDV from the serum of younger persistently infected cattle.[7,29]

Immunohistochemistry test. The IHC test is conducted on skin biopsy specimens (ear notches) collected from animals of any age, fixed in formalin, and submitted to the diagnostic laboratory.[32–34] The fixed skin specimens are sectioned, stained, and examined for the presence of BVDV antigen. The IHC test, like the VI test, has excellent sensitivity and specificity.[34] Sensitivity of IHC studies is not affected by the presence of maternal antibody, so calves of any age, including newborn calves, may be tested.[34,35]

Polymerase chain reaction assay. The PCR assay may be used to test individual animals (serum, whole blood, or skin samples) or to screen entire herds by testing pooled samples such as bulk tank milk or pooled serum samples for the presence of carrier cattle.[36] The BVDV PCR assay is highly sensitive, but a potential complication with the assay is lack of test specificity, so that false positive results are possible from nonspecific reactions with contaminating viral RNA (unpublished observation). It is therefore advisable to confirm positive BVDV PCR assay results with VI tests.[35]

Enzyme-linked immunosorbent assay. The ELISA may be used to test individual blood samples for the presence of BVDV antigen. The antigen-capture ELISA compares closely with virus isolation techniques for detection of persistently infected cattle using blood samples routinely submitted for BVD diagnosis. The test format is adapted to a microtiter plate assay, which permits efficient testing of large numbers of serum samples.[31,37]

Aborted Fetuses

Diagnosis of BVDV as the cause of abortion is not unequivocal, because fetal infection may not result in abortion. Therefore, the presence of virus, viral antigen, or BVDV antibody in an aborted fetus does not confirm that BVDV was the cause of abortion.[28] The entire fetus should be submitted to a diagnostic laboratory for complete testing because of the complexity of the factors to be considered in conclusively establishing BVDV infection as the cause of abortion or, conversely, in ruling out BVDV infection as the cause of abortion. Diagnosis of BVDV as the cause of abortion is based on evidence of BVDV infection of the fetus (presence of virus, antigen, or RNA in tissues, or antibody in serum or exudates), in conjunction with clinical confirmation of microscopic lesions, most often in fetuses aborted before 4 months of gestation. Microscopic lesions attributable to fetal BVDV infection include a necrotizing inflammatory reaction with mononuclear cell infiltration in several tissues.[38] Other features may include lymphoid depletion of the cortex of the thymus, precocious development of secondary lymphoid tissue, and peribronchiolar lymphonodular hyperplasia. The cerebellum is affected with necrosis and depletion of cells and infiltration of mononuclear cells. Microfocal lesions may be seen in the oral mucosa and in the skin. Skin lesions are characterized by hyperkeratosis and parakeratosis. BVDV antigen may be deposited in lymphoid tissues and in the cerebellum.[9] Demonstration of rising BVDV antibody titers in paired serum samples from dams may be not be possible. This is because antibody titer may have already increased at the time of abortion because of the time lag from infection of the dam to abortion. Identification of BVDV in a fetus in the absence of lesions provides useful information regarding the temporal occurrence of BVDV within the herd.

Congenital Developmental Defects in Term Calves

Diagnosis of BVDV as the cause of congenital developmental defects in term calves is based on evidence of transplacental BVDV infection in combination with presence of characteristic clinical signs and gross or microscopic lesions. Evidence of transplacental BVDV infection is obtained by culture of BVDV or detection of BVDV antigen or RNA. Antibody in serum collected from a calf before it has ingested colostrum also constitutes evidence of transplacental BVDV infection. Calves born with cerebellar hypoplasia have difficulty standing and exhibit a wide-based stance, and are ataxic. Blindness may result from congenital defects of the eye. Ophthalmic examination may be performed to reveal the presence of cataracts. Calves may be born weak and undersized subsequent to fetal growth retardation due to BVDV infection.[28] Gross lesions involving the nervous system, eye, immune system, integumentary system, musculoskeletal system, or respiratory system are described in greater detail earlier in the chapter. It is prudent to exercise caution in attributing these lesions to BVDV without demonstrating virus or viral antibody, because other causes for many of these lesions exist. Unfortunately, the virus frequently is cleared by the time calves are born. Presuckle serum is useful for diagnostic applications.

Late-Gestation Transplacental Infections

The presence of BVDV antibody titers in serum collected from normal term calves before they have ingested colostrum indicates that infection occurred late in gestation after the fetus developed immunocompetence.

Screening Herds without a History of BVDV Infection

A herd that does not have a history of BVDV infection may be screened to determine if BVDV infection is active in the herd (i.e., determine if animals with either acute or persistent infections are present). One screening approach that limits the expense of testing, as well as labor requirements, is testing for the presence of antibodies to BVDV in a representative subset of nonvaccinated, sentinel cattle[39] that are at least 8 months old.[40] The presence of BVDV antibodies in any of these animals indicates that one or more acutely or persistently infected animals are present in the herd.[40,41] The absence of BVDV antibodies indicates that carrier cattle that shed virus, both acutely and persistently infected animals, are not present—the herd is BVDV-free. PCR assays of the somatic cells of bulk-tank milk is another approach that has been used in dairy herds to screen for evidence of carriers with persistent BVDV infection among lactating cows.[36]

Prevention and Control

The goal of a BVDV control program is to prevent fetal infection to eliminate BVDV-associated reproductive losses and the birth of persistently infected calves. Control of BVDV infection is best achieved by avoiding persistently infected carrier cattle and acutely infected cattle, and by maintaining sound immunization practices. Elimination of persistently infected carriers from the herd is accomplished by testing the herd and by closing the herd to incoming animals that are potentially persistently infected carriers or acutely infected, transient virus shedders. Identification and removal of persistently infected cattle require accurate herd-based diagnostic laboratory testing.[42]

Detection and Removal of Persistently Infected Carrier Cattle

Removal of persistently infected cattle from a herd and prevention of reintroduction of persistently infected cattle into a herd are essential herd management procedures because persistently infected cattle are the primary source of infection for a herd. Selection of replacement breeding cattle on the basis of performance effectively eliminates some persistently infected cattle from the herd on the basis of poor growth rates. Other persistently infected animals may be eliminated from herds because of the shortened lifespan sometimes associated with persistent infection in cattle. Some persistently infected cattle, however, may have normal growth rates[21,39] and normal lifespans and accordingly be retained in the breeding herd. Consequently, the importance of using laboratory tests to ensure detection and removal of all persistently viremic cattle from a herd is clearly evident. Seed stock producers, especially breeders of purebred cattle, should be strongly encouraged to test their animals and remove persistently infected carrier animals from their herds.[35]

The greatest proportion of persistently infected animals in BVDV-infected herds are calves younger than 6 months of age.[43] Initially, calves are tested, rather than dams, because if calves are tested, information about the persistent infection status of the calves and about that of their dams is obtained simultaneously. This is because persistently infected dams always give birth to persistently infected calves. IHC testing of skin biopsy specimens (ear notches) is recommended for this procedure. Testing and removal of persistently infected calves from the herd must be completed before the breeding season begins, to prevent contacts of persistently infected calves with pregnant cows, so as to prevent transplacental infection, production of persistently infected calves, and perpetuation of infection within the herd. In addition to testing calves, replacement heifers, cows not calving, bulls, and dams of any calves that test positive must be tested.[35]

Biosecurity

Before implementation of an extensive BVDV testing program in a herd, the potential for re-exposure of a BVDV-free herd to BVDV infection after completion of a persistently infected carrier testing and removal program must be considered. Introduction of BVDV infection into a herd may occur by contact with cattle from other herds or with addition of animals to herds. Requirements for a BVDV biosecurity program are that all purchased cattle be tested for persistent infection status or originate from a BVDV-free herd. Purchased replacement animals should be isolated and tested before being added to the herd to avoid introduction of acutely-infected animals. The offspring of purchased pregnant replacement cattle must also be tested to confirm their BVDV persistent infection–free status before being added to the herd.[40,44] Seed

stock producers are obligated to maintain a BVDV-free herd by maintaining strict biosecurity practices, including testing of all animals in their herd to warrant BVDV-free status.[35]

Vaccination and Immunity

The goal of immunization is to prevent infection of target organs such as the fetus. This is accomplished by inducing both the B and T cell arms of the immune system. Free virus is inactivated by the B cell arm of the immune response, by neutralization of BVDV infectivity by immunoglobulin, and secondarily by aggregating virions and enhancing clearance. Infected cells that have potential to release infectious virus are eliminated by T cells.[45]

Optimally, vaccines should provide broad cross-protective immunity that protects the fetus against all field strains of BVDV. Vaccines do provide significant protection against fetal infection, which will limit reproductive disease, including production of persistently infected calves. Vaccines do not provide absolute fetal protection, however. Results of experimental challenge vaccine studies have shown that no BVDV vaccine can induce complete fetal protection, and birth of persistently infected calves in vaccinated cows has been reported in the field. Consequently, vaccination should not be relied on completely to provide protection against fetal infection. Management practices also should be implemented to identify persistently infected carrier cattle and eliminate them from the herd, and to avoid exposure to BVDV infection.[35,46]

Modified live virus vaccines against BVDV activate systemic and local, humoral and cell-mediated immune responses. Immunity induced by modified live virus vaccine generally is more cross-reactive than that induced by inactivated vaccines. Cross-reactivity is important for BVDV immunity because the potential for antigenic variation exists. Other advantages of modified live virus vaccines include longer duration of immunity and a reduced requirement for repeated administration of vaccine. Disadvantages of these vaccines are immunosuppressive properties and the potential to cause severe fetal anomalies and disease attributable to vaccine contamination with adventitious BVDV. Another disadvantage is the potential for restoration of virulence of the virus during infection. Experimental challenge-exposure studies have demonstrated a reasonable degree of protection against fetal infection using a modified live virus vaccine.[47]

Inactivated vaccines are neither immunosuppressive nor fetopathogenic. Inactivated vaccines also offer the advantage of immunization with minimal risk of infection. In general, disadvantages of inactivated BVDV vaccine may be a need for increased frequency of administration due to a weaker neutralizing antibody response and shorter duration of protection.

Timing of vaccination. The best general recommendation for control of BVDV disease includes avoiding addition of replacement animals that are persistently or transiently infected, avoiding purchase of pregnant cattle, removal of carrier cattle from the herd, and adherence to a vaccination schedule based on use of both modified live virus and inactivated BVDV vaccines. It must be recognized that live, replicating vaccines (i.e., modified live virus vaccines) have certain inherent properties (see preceding discussion of benefits of modified live virus vaccines versus inactivated vaccines) that may enable them to induce more complete protection against transplacental infection. Therefore, it may be wise to recommend vaccination of unstressed, healthy heifers, isolated from pregnant cows, with modified live virus vaccine. All replacement heifers should be vaccinated twice with modified live virus before breeding. In non-vaccinated animals, modified live virus vaccines should be administered three estrous cycles (i.e., 2 months) before breeding. Administration of inactivated vaccines to heifers before breeding should be timed so that maximal responses are achieved. Booster vaccinations should be administered in accordance with the vaccine manufacturer's recommendation.

It may be difficult to time vaccination in a dairy herd to avoid use of modified live virus vaccines on premises with pregnant animals, because dairy cows typically are at various stages in their reproductive cycles. Beef calves, weaned at 5 to 7 months of age, typically are seronegative or have low titers of maternal antibody at weaning time.[7] Thus, calves should be immunized before weaning so that they are protected at weaning when they enter concentration points at which a high risk of infection exists.[46]

BOVINE HERPESVIRUS-1 (INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS) INFECTION

Bovine herpesvirus-1 (BHV-1) is well recognized as a pathogen that infects the respiratory and reproductive tracts and also infects the fetus, potentially leading to abortion. BHV-1 infects cells of the upper respiratory tract, causing rhinitis, conjunctivitis, and tracheitis. Respiratory tract infections with BHV-1 also may contribute to establishment of bacterial bronchopneumonia by impairing host defenses, such as by diminishing lung clearance mechanisms and by immunosuppression. If infection occurs in nonimmune pregnant females, systemic infection, fetal infection, and abortion are the likely consequences. Genital infections may result in development of pustular vulvovaginitis in females or balanoposthitis in males. Genital infections, characterized by formation of variable numbers of small nodules, vesicles, focal erosions, or ulcers visible on inflamed mucosal membranes, occur transiently and resolve spontaneously in 1 to 2 weeks.

Important Biologic Properties of the Virus

BHV-1 is a member of the family Herpesviridae, subfamily Alphaherpesvirinae. In addition to causing a range of clinical diseases, it also can establish latent infections localized in trigeminal and sacral ganglia.[48] Latent BHV-1 can become reactivated under certain circumstances such as stress or after corticosteroid treatment.[48] Vaccination with most modified live virus vaccines has the potential to produce latent infections. Because of viral latency and reactivation, cattle that recover from BHV-1 infec-

tions may serve as a source for future infection of unexposed cattle. BHV-1 is fairly resistant to environmental influences and can survive for 5 to 13 days in warmer environments, but because the virion is enveloped, it is susceptible to most disinfectants.[49] Only one serotype of BHV-1 is recognized.

Infection by BHV-1 is transmitted by direct contact with upper respiratory, conjunctival, or genital tract mucous membranes. Infected animals shed virus from respiratory mucous membranes and secretions, or genital mucous membranes and secretions, for 8 to 16 days after exposure.[49] The virus is present in all fetuses aborted as the result of BHV-1 infection, and these fetuses can serve as a source for transmission of disease.[50,51] Venereal transmission and the use of contaminated semen or instruments during artificial insemination are the primary means for transmission of these genital infections. BHV-1 can be isolated from the semen of exposed but clinically normal bulls, and this should be considered when exposed bulls are used either for natural breeding of unexposed cows or as semen donors.[48]

Fetal Infection and Abortions

When BHV-1 respiratory tract infections occur in nonimmune pregnant females, the likely outcomes are viremia and subsequent fetal infection and abortion.[50] Exposure of multiple susceptible animals in a herd can result in abortion storms, with as many as 25% to 60% of cows in a herd aborting.[51–53] Sporadic abortions also may be seen, particularly in herds with a previous history of vaccination or exposure. Abortions also can occur when pregnant cattle are vaccinated with conventional modified live virus vaccines.[54]

BHV-1 abortions may occur at any gestational stage, but naturally occurring abortions are most common between 4 and 8 months of gestation.[51–53,55] Aborting cattle may be subclinically infected or exhibit overt clinical disease. When clinical signs are present in aborting cows, they usually manifest as respiratory tract disease or conjunctivitis.[55] Abortions are rarely seen in conjunction with infectious pustular vulvovaginitis.[50] Abortions often do not occur for several weeks after appearance of clinical signs in the dam. The incubation period in one group of pregnant heifers, experimentally infected intravenously, ranged from 17 to 85 days.[56] The mechanism for this latent period between maternal exposure and abortion is unknown, although some evidence suggests that the virus may reside in the placenta for extended periods before infecting the fetus proper, without causing abortion.[51]

Diagnosis of Abortion

BHV-1 infection of the fetus results in rapid fetal death (24–48 hours), but expulsion is delayed for up to 7 days, with consequent autolysis of tissue of variable degree. The placenta often is retained. Gross changes in the aborted fetuses often are obscured by autolysis, but tiny (1- to 3-mm-diameter) white-tan foci may be evident on the surface of liver and lung. Red-tinged serous fluid in body cavities and red color of fetal tissues reflecting the autolysis usually are evident. The placenta may be edematous.[52]

BHV-1 abortions can be confirmed by immunohistochemical tests and microscopic examination of fetal tissues. Microscopically, scattered foci of necrosis in several organs may be present, which can lead to a presumptive diagnosis of BHV-1 abortion. In particular, foci of necrosis may be found in the liver, spleen, adrenal glands, lung, kidneys, and placental cotyledons. Herpesviral intranuclear inclusions may be present in cells adjacent to necrotic foci but usually are masked because of autolysis. Little or no inflammatory cell infiltrate is found in fetal tissues, reflecting the rapidly lethal effects of BHV-1 fetal infection. The diagnosis can be confirmed either by detection of BHV-1 viral antigen or viral nucleic acid or by isolation of the virus from fetal tissues. Viral isolation may be difficult, depending on the degree of fetal autolysis; placental cotyledons are the preferred tissue for virus isolation attempts if autolysis of fetal tissue is extensive.[51]

The BHV-1 antigen can be detected in cryostat tissue section–fluorescent antibody tests (especially kidney and adrenal) and from paraffin-embedded formalin-fixed tissues (especially liver, lung, kidney, adrenal, and placenta) by immunohistochemistry studies.[57] Viral nucleic acid has been detected in aborted fetuses by in situ hybridization[58] and by PCR assay.[59] Determination of BHV-1 antibody titers on paired maternal serum samples is of little help in diagnosing BHV-1 abortions. Most abortions occur several weeks after infection of the dam, so increases in antibody titers will have occurred before abortion. Maternal antibody titers indicate exposure, but without demonstration of rising antibody titers, it is not possible to confirm recent BHV-1 infection.

Prevention and Control

Prevention and control of BHV-1–induced abortion in herds are achieved primarily by implementation of sound biosecurity practices and by vaccination. Biosecurity practices should include control of movement of new stock into a breeding herd to eliminate the possible introduction of BHV-1 into a susceptible herd. Screening of semen used in artificial insemination for BHV-1 contamination and selection of seronegative donor bulls are recommended measures to prevent venereal transmission.[60] Eradication programs have been established in certain European countries, such as Switzerland, Denmark, and the Netherlands.[61]

Vaccination commonly is practiced to prevent and control BHV-1 infection because of the high prevalence of BHV-1.[51] Numerous BHV-1 vaccines are commercially available and include both modified live virus and inactivated vaccines. Manufacturers' recommendations should be followed to achieve optimal immune responses and to avoid potential adverse effects associated with their use. Most modified live virus vaccines can cause all of the manifestations of BHV-1 infection in the bovine reproductive tract.[62] Because of the risk of vaccine-induced abortions and exposure of susceptible dams, most modified live virus vaccines are not recommended for use in pregnant cattle or calves suckling pregnant

cows. Inactivated vaccines are safe for use in pregnant cattle. If modified live viruses are routinely employed, the timing of injections can be scheduled to reduce the risk of vaccine virus–induced abortion or transmission of virus to susceptible animals. In beef herds, heifers may be vaccinated before the beginning of breeding season. In dairy cattle, the simplest means of reducing such risk is to vaccinate heifers only before breeding (4–6 months of age, and again at 8–12 months) and to vaccinate postpartum heifers and cows at the time of routine postpartum examinations. These animals are not pregnant and tend to be housed with like animals. Furthermore, routine vaccination should result in development of a "herd immunity," so that likelihood of transmission of vaccine virus to susceptible cattle is reduced.[63]

BLUETONGUE VIRUS INFECTION

Bluetongue virus is an orbivirus that infects cattle and sheep; in North America, it is transmitted by the biting midge, *Culicoides variipennis*.[64] The midge becomes persistently infected with bluetongue virus and may transmit virus for several weeks.[65] Bluetongue virus infection of cattle is common in endemic areas of the world, which correspond to the geographic distribution of the vector. Bluetongue virus is not contagious, and vertical transmission is not important, so perpetuation of the virus in nature is dependent on continuous cycling of virus between the insect vector and susceptible ruminant animals.[66] Cattle are considered to be natural reservoir hosts of bluetongue virus. Although bluetongue virus is common in cattle in endemic areas, bluetongue disease is rare.[67] After onset of infection, cattle may be viremic for up to several weeks, during which time infected animals may act as viral amplifiers and reservoirs for the transmitting *Culicoides* vector.[65] Although viremias may be prolonged, they are not persistent, nor does this agent cause immunotolerance.[67] Prolongation of viremia in cattle, which facilitates infection of the insect vector, probably is the result of the strong association of bluetongue virus with erythrocytes, which may protect virus from elimination by neutralizing antibody.[65]

Fetal Infection and Abortions

Bluetongue virus fetal infection of cattle and sheep can occasionally result in abortion, but teratogenesis is more common.[68] Naturally occurring bluetongue virus fetal infection and abortion have been reported in cattle, but only in countries in which modified live virus vaccines have been used. Vaccine strains of bluetongue virus are the likely cause of naturally occurring fetal infection, because studies in pregnant sheep have shown that bluetongue virus crosses the placenta and produces fetal malformation only after the virus has been altered by adaptation to cell culture.[68]

When rare fetal infections with bluetongue virus do occur, they mimic results from experimental infections. Infection of susceptible heifers or cows during early gestation (during the first 90–100 days of gestation) may result in fetal death (by either resorption or abortion). Fetal infection between 75 and 100 days of gestation results in stillborn fetuses or in the birth of weak calves or calves with cerebral malformations.[69] Central nervous system abnormalities due to bluetongue virus infection in term calves may include hydranencephaly or cerebral cysts.[67–69] Cerebral malformations do not occur in fetuses infected after 150 days of gestation. These late-term fetal infections may have no effect on gestation or may result in premature births. The infected calves may have no visible lesions or a mild nonsuppurative encephalitis.

Diagnostic and Control Considerations

In most instances, infected fetuses surviving beyond midgestation have detectable fetal or precolostral antibodies to the bluetongue virus, but the virus is no longer present.[67–69] The virus is difficult to isolate, and freezing of infected tissue destroys the virus. The virus is rarely if ever isolated from term fetuses if infection occurred before 150 days.[69] Virus may be present in the semen of seropositive bulls, but only when they are viremic. Bluetongue virus in semen is associated with the presence of contaminating erythrocytes or mononuclear cells that carry virus; the virus is not found in spermatozoa.[67] International regulations prohibit movement of livestock and germplasm from countries harboring animals with bluetongue viruses to countries considered virus-free.[67] U.S. livestock industries incur significant losses each year because of these trade restrictions, even though supporting evidence for transmission of bluetongue virus between countries, by either semen or embryos, is lacking.[67]

Vaccines are available, but owing to the rare occurrence of fetal infection or clinical disease in cattle, their use even in endemic regions seems unwarranted. Cows develop immunity to the infecting serotype but remain susceptible to infection by other serotypes.[69] Because bluetongue virus is transmitted by *Culicoides* spp., the modified live virus in the vaccine also may be transmitted among animals by the insect vector; thus, if vaccines are used, administration of vaccines should be limited to times of the year when vectors are inactive.[67]

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CHAPTER 51

Protozoal Abortion in Cattle

BRUCE ABBITT and D. OWEN RAE

Ruminant protozoal abortifacients include *Tritrichomonas foetus* and *Neospora, Toxoplasma,* and *Sarcocystis* spp. The latter three genera all are members of the family Sarcocystidae. The genus *Sarcocystis* is composed of 130 species of cyst-forming coccidians with differences in life cycle and pathogenicity.[1] All Sarcocystidae species with known life cycles exploit natural predator-prey relationships. Sexual reproduction takes place in the intestine of the predator, resulting in fecal passage of oocysts that are orally infective for the prey species (intermediate host); asexual reproduction takes place in tissues of the prey species, which are then orally infective for the predator species (definitive host). For example, the several *Sarcocystis* species that infect the bovine—*Sarcocystis cruzi, Sarcocystis hirsuti,* and *Sarcocystis hominis*—have raccoons and canids (dog, wolf, coyote, fox), felids, and primates (humans, monkey), respectively, as definitive hosts. Felids act as the definitive hosts for *Toxoplasma gondii,* with almost all warm-blooded animals potentially serving as intermediate hosts. Infection by *T. gondii* also can be maintained in successive generations of two common intermediate hosts (rats and mice) by congenital (dam-to-daughter) transmission.[2,3]

The life cycle of *Neospora* has only recently been determined but has been shown to follow a pattern similar to that for other species of Sarcocystidae (Fig. 51-1). Oocysts were found in dog feces,[4] and it appears that the dog can play the role of both an intermediate and a definitive host. Other intermediate hosts include ruminants and canids.[5] This agent is maintained in successive cattle generations by congenital (dam-to-daughter) transmission. It also is increasingly clear that *Neospora caninum* is the most important abortifacient agent in this group.

NEOSPORA CANINUM

N. caninum was discovered in 1984 as the cause of congenital infection of the central nervous system in dogs, and in 1988 the organism was isolated and named.[6] Slight ultrastructural and antigenic differences exist between tissue phases of *T. gondii* and *N. caninum*.[7,8] Comparison of ribosomal RNA or DNA sequences indicates a close phylogenetic relationship but distinct species in analysis by random amplified polymorphic DNA–polymerase chain reaction.[9–11] The definitive host of *N. caninum* is the dog[4]; wild canids are intermediate hosts and may be implicated as definitive hosts with further study.[12,13] Researchers have found antibodies in naturally exposed water buffalo, coyotes, red foxes, and camels, suggesting that these animals are natural intermediate hosts.[5] Congenital infection by *N. caninum* has been documented in many species. Naturally occurring congenital infections were first documented in dogs[14] and cattle.[6] Further study suggests that natural and experimental congenital infection occurs in horses, sheep,[15] goats,[16] deer,[17] and mice.[18] As has been proved for *T. gondii,* it is possible that infection is acquired congenitally, by ingestion of oocysts (fecal contamination of feed), or by contact (nasal, ocular, or oral) with cysts or tachyzoites.[19]

Since the 1960s, there have been many reports of abortion in cattle due to unidentified protozoa, often tentatively assumed to be either *Sarcocystis* spp. or *T. gondii*.[20] Retrospectively, most cases involved microscopic lesions that are now recognized as virtually diagnostic for fetal infection by *N. caninum.* These microscopic lesions are most consistently nonsuppurative encephalitis with foci of necrosis and gliosis, and nonsuppurative myositis, hepatitis, and myocarditis. Protozoa are rarely found by microscopic evaluation of hematoxylin-eosin (H&E)-stained sections from affected fetuses.[21] With use of immunohistochemistry studies,[22] however, protozoa can be visualized in most fetuses with lesions typical of protozoal abortion.[7]

Reported cases indicate worldwide distribution of this abortifacient in cattle. Cases have been documented in Canada,[23,24] the United States,[25,26] the Netherlands,[27] New Zealand,[28] South Africa,[29] Australia,[30,31] Mexico,[32] England and Wales,[33] and Japan.[34] Continued investigation during the past decade validates the worldwide distribution of infection in dairy and beef cattle globally.[5] In California[21,35] and similarly in New Zealand,[28] *Neospora* has been diagnosed as the cause of abortion in approximately 20% of fetuses from dairy cattle submitted to diagnostic laboratories and the cause of abortion in approximately 44% of all fetuses originating from California dairy herds with a prior history of *Neospora* abortions.[36] In the midwestern United States, a survey of 655 aborted bovine fetuses submitted to a state diagnostic laboratory revealed 2.7% to be the result of *Neospora* infection; of interest, 19 of 20 of the *Neospora*-infected fetuses represented dairy breeds and 12% of submitted fetuses from dairy cows were diagnosed with *Neospora* infections.[37]

The dog, as the definitive host of *Neospora,* contributes to the horizontal transmission of the agent and thereby the establishment of infection.[4,38–42] Infection can then be propagated by vertical transmission in the bovine through multiple generations.[41–43] This horizontal introduction by a definitive host combined with vertical propagation from the infected intermediate host helps to explain the high prevalence and worldwide distribution of *Neospora* abortion. Because the dog generally has both worldwide distribution and a common association with

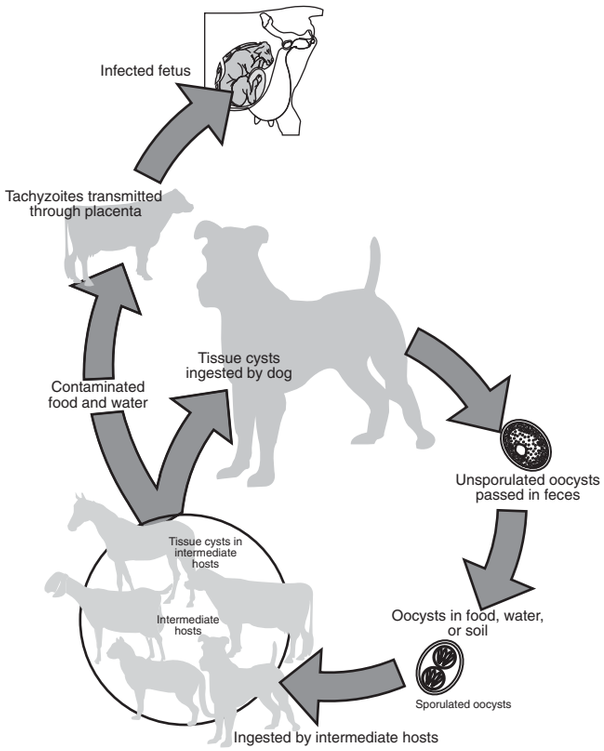

Fig. 51-1 The life cycle of *Neospora caninum*.

dairies and livestock enterprises, the likelihood of cattle exposure to infection may be increased. Of interest, naturally occurring abortions and congenital infection with *Neospora* also have been documented in sheep,[44] goats,[16] horses,[45] and black-tailed deer.[17]

Neospora-induced abortions typically occur during the early second trimester[25,26,28,35–37] but can occur throughout gestation.[28,32,35] On gross examination, aborted fetuses often are moderately or severely autolyzed, but microscopic diagnosis usually is possible because of persistence of both characteristic lesions and protozoa.[28,32,33,43] Desirable samples for submission to the diagnostic laboratory include the entire fetus and placenta or samples from the brain, heart and liver, as well as body fluids or blood serum.[5] Although autolysis may present some difficulties in preparation of samples, semiliquid brain tissue should be fixed in 10% buffered neutral formalin for histologic and immunohistochemistry examination.[5] Cows aborting *Neospora*-infected fetuses are not clinically sick, and in one report, conception rates to artificial insemination were not adversely affected by a history of *Neospora* abortion.[32]

A sensitive and specific serologic test is available,[46] and serum from congenitally infected calves is positive before intake of colostrum.[6,46] Term calves with serologic and/or microscopic evidence, or both, of *Neospora* infection have been reported to have enhanced survivorship during the preweaning period, to be essentially clinically normal, or to have various degrees of central nervous system impairment.[6,30,47–50] Serologic studies in California drylot dairies reveal that herd infection rates can be estimated by serologic testing of calves before colostral intake, because congenital infection occurs in 78% to 80% of seropositive cows.[47] Repeated serologic testing of individual cows from these dairies reveals an apparent lack of new infections (i.e., serologically positive cows remain positive and serologically negative cows remain negative). Furthermore, the percentage of serologically positive cows that abort is approximately twice that of seronegative herdmates.[47] The absence of seroconversion (from seronegative to seropositive status in adult animals) in these herds strongly indicates maintenance of herd infection by the primary means of placental or congenital transmission.

Congenital transmission may explain the endemic pattern of abortion observed in herds suffering annual abortion rates exceeding 5% for several years.[51] Epidemic patterns of abortion, however, also may be seen—that is, a high percentage of abortions may occur during a relatively short period of time.[25,52] Several studies have shown a three- to fourfold increase in abortion risk for seropositive cows compared with that for seronegative herdmates.[53,54] Repeat abortion in the same cow has been observed in an infected herd[32] and was proved in four cows in a California dairy herd.[6] Furthermore, the cow with a history of previous abortion as a result of *N. caninum* infection has up to a 5.7 times greater risk of abortion in the subsequent pregnancy.[55] The serologic findings in California drylot dairies suggest that repeat abortions are caused by recrudescence of latent infection, although acquisition of a new infection also is possible.

TOXOPLASMA GONDII

T. gondii is a significant, well-documented cause of abortion and congenital infection in sheep and goats; the clinical manifestations are very similar to those of *Neospora* infection in cattle.[56–61] This similarity is not surprising in view of the close phylogenetic relationship between these organisms.[10,11] *T. gondii* previously has been suspected as a cause of congenital infection with associated neurologic effects or abortion in cattle,[20] but the cause in such cases is now disputed or proved to have been *Neospora*.[49,62] Additionally, *T. gondii* has rarely been isolated from bovine tissues, and experimental infection of pregnant cows by oral administration of *T. gondii* oocysts does not result in abortion.[62]

SARCOCYSTIS

The clinical signs of acute sarcocystosis in cattle include pyrexia, ptyalism, enlarged lymph nodes, anemia, and abortion; these have been reproduced by oral administration of *S. cruzi* oocysts.[63–66] Experimentally induced abortions were characterized by a general absence of protozoa within aborted fetuses. By contrast, protozoal schizonts are readily found in endothelial cells in multiple organs of the dam, indicating that abortion is due to systemic illness of the dam and not protozoal infection of

the fetus.[65,66] Regardless, the paucity of clinical reports of acute bovine sarcocystosis suggests this to be a rare clinical occurrence. In the few published case reports, *Sarcocystis*-induced abortion was characterized by an absence of illness in the dam and presence of protozoa in multiple organs of the fetus. The location of protozoal schizonts within endothelial cells and immunoreaction of the schizonts confirm *Sarcocystis*.[67,68] In surveys in England and Wales[33] and in California,[7] however, either 0 (England and Wales) or 4% (California) of aborted bovine fetuses with microscopic lesions typical of protozoal abortion were infected by *Sarcocystis;* the remainder were infected by *Neospora*. In summary, *Sarcocystis* is a proven, but rare, bovine abortifacient.

Prevention and Control

The ruminant protozoal abortifacients discussed in this chapter all are members of the family Sarcocystidae. Prevention should include measures that disrupt the predator-prey life cycle of these protozoa, thereby interrupting horizontal transmission. Carcasses and placentas should be disposed of in a manner to prevent consumption by the definitive host animals, and feed and water should be handled, stored, and supplied in a manner to prevent or minimize fecal contamination. The vertical transmission of *N. caninum* through chronically infected cows requires that these be identified and removed from the herd and that introduction of infected replacement heifers be prevented or limited. The ability to screen cattle for presence of *Neospora* by serologic tests (both adults and newborn calves before intake of colostrum) provides a means of selecting noncarriers for replacements and eliminating carriers from within a herd.

Treatment of carriers in vivo has not been shown to be effective at present[69]; various drugs have been shown to inhibit growth of *N. caninum* in vitro,[70] and some treatment regimens have been evaluated in *N. caninum*–infected puppies.[71] Management of these agents should focus on prevention and control, not treatment.

Research directed at producing an effective immunizing agent has had limited success to date. Killed *N. caninum* or its products have been injected into prepartal mice and shown to block transplacental transfer of the agent,[72,73] but in cattle, clear evidence of vaccinal prevention of *N. caninum* abortion is lacking.[74] Protective immunity seems to develop after primary exposure, reducing or potentially preventing subsequent abortion.[44,75,76] Particular challenges, however, arise in conferring protective immunity to animals already infected naturally,[77,78] and in preventing vertical transmission.[75] Although investigation of biologic, pathophysiologic, and immunologic properties of *N. caninum* continues, rudimentary products are being employed in cattle populations to reduce the impact of the agent. For example, a recent field trial showed a 50% reduction in abortions of dairy cows given commercial *N. caninum* vaccine, as compared with cohort control cows (11% and 21%, respectively).[79]

A clearer understanding of protozoal abortion is speedily emerging—specifically, *N. caninum* abortion. Continued investigation is anticipated to reward us with a better understanding of its pathophysiology, control, and prevention.

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CHAPTER 52

Epizootic Bovine Abortion (Foothill Abortion)

ROBERT H. BONDURANT, MARK L. ANDERSON, JEFFREY L. STOTT, and PETER C. KENNEDY

HISTORY AND ETIOLOGY

Epizootic bovine abortion (EBA), also known as **foothill abortion**, is a distinct, tick-transmitted disease of pregnant cattle that graze foothill and mountain pastures of California and adjacent states.[1] Endemic areas often are bushy or wooded regions, typically dry chaparral or oak-studded environments from 500 to 7000 feet above sea level, and are the habitat of the argasid tick vector *Ornithodoros coriaceus*[2] (Fig. 52-1). Heifers confined to permanent (irrigated) pastures within endemic areas rarely if ever experience EBA.

A causative agent for EBA has been sought for more than 50 years.[3] Research through the mid 1980s suggested several different agents, including chlamydiae *(Chlamydophila),* viruses, and spirochetes; however, recent breakthroughs, using molecular techniques, chemical and immunohistologic stains, and other methods, have implicated a bacterial agent.[3–6] Although the disease has a limited geographic distribution, it is a major cause of abortion, neonatal mortality, and economic losses in endemic areas. It also is of interest to the veterinary scientist because of its unusual presentation, long latent period, and pathologic features.

NATURAL HISTORY

Typically, only heifers, or adult cows brought for the first time into endemic areas during or after the breeding season, are susceptible to the disease. Abortions, which usually occur sporadically but may come in dramatic "storms," result in the loss of late-term fetuses (6 to 9 months of gestation). Birth of live but unthrifty calves also may occur as part of an EBA outbreak. If the herd is not moved from one calving season to the next, heifers and cows that abort an EBA fetus in one season tend not to suffer EBA again in the following year.

In the 1960s, the geographic distribution of the soft-bodied tick, *O. coriaceus* (see Fig. 52-1), was shown to

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A causative agent for EBA has been sought for more than 50 years.[3] Research through the mid 1980s suggested several different agents, including chlamydiae *(Chlamydophila),* viruses, and spirochetes; however, recent breakthroughs, using molecular techniques, chemical and immunohistologic stains, and other methods, have implicated a bacterial agent.[3–6] Although the disease has a limited geographic distribution, it is a major cause of abortion, neonatal mortality, and economic losses in endemic areas. It also is of interest to the veterinary scientist because of its unusual presentation, long latent period, and pathologic features.

NATURAL HISTORY

Typically, only heifers, or adult cows brought for the first time into endemic areas during or after the breeding season, are susceptible to the disease. Abortions, which usually occur sporadically but may come in dramatic "storms," result in the loss of late-term fetuses (6 to 9 months of gestation). Birth of live but unthrifty calves also may occur as part of an EBA outbreak. If the herd is not moved from one calving season to the next, heifers and cows that abort an EBA fetus in one season tend not to suffer EBA again in the following year.

In the 1960s, the geographic distribution of the soft-bodied tick, *O. coriaceus* (see Fig. 52-1), was shown to

Fig. 52-1 Adult engorged *Ornithodoros coriaceus* tick, the vector of epizootic bovine abortion. (Courtesy of M. Oliver.)

Fig. 52-2 Epizootic bovine abortion fetus, with petechiae on the ventral surface of the tongue and oral mucosa. (Courtesy of Dr. P. C. Kennedy.)

coincide with the known areas in which EBA had been reported.[2] The most common hosts of this ectoparasite are deer and cattle. Experimental feeding of the tick on susceptible pregnant heifers was shown to reproduce the disease.[2,7] On the basis of this and other work, the role of *O. coriaceus* as a vector of the EBA agents has been accepted. Various agents have been isolated from this tick, but none of these agents has reliably reproduced EBA.[3] Experimental work has shown that the thymus of EBA-aborted fetuses reliably transmits the disease when extracts are inoculated into susceptible pregnant heifers. In recent studies, the "thymic agent" was shown to be nonfilterable (e.g., infectivity was removed by filtration); it also was destroyed by sonication, and its pathogenicity was ablated by antibiotic treatment.[4] Although no agent has been reliably cultivated from the thymus, molecular techniques that amplify microbial nucleic acids (polymerase chain reaction [PCR] assays) identified a deltaproteobacterial agent, specifically of the Myxococcales order.[5] This order is notorious for failure of organisms to grow in in vitro culture. So, although the demands of classic Koch's postulates have not been met, a majority of guidelines that have been suggested for DNA sequence–based determination and incrimination of a presumptive microorganism in disease have been satisfied.[8]

CLINICAL SIGNS AND FINDINGS

Heifers or cows that have been either naturally or experimentally exposed to EBA show no overt signs of illness at any time after exposure. Minor elevations in total leukocyte counts about 1 week following tick feedings have been noted. The interval from exposure to abortion is unusually long, typically 3 to 4 months, with nearly all abortions coming in the third trimester. When fetuses are expelled, they usually are in a relatively fresh (i.e., nonautolytic) state. Serum from aborted EBA fetuses shows markedly elevated immunoglobulin concentrations, especially immunoglobulin G (IgG) (150–800 mg/dl; geometric mean, approximately 300 mg/dl), and in some instances, the immunoglobulin levels can approach those of colostrum-fed neonates (>1 g/dl).[7,9] We are not aware of another disease entity that routinely stimulates such high levels of bovine fetal immunoglobulin.

The chronic nature of the disease process in the fetus and the late stage of gestation suggest that the heavily stressed fetus secures its own delivery through cortisol release, and that some calves may survive parturition yet succumb in the neonatal period. Often, heifers or cows in which abortion has occurred commence lactation; in any case, they tend to show few lasting effects of the infection, and they generally are able to conceive during the following breeding season, provided that the abortive delivery proceeded without complications.

PATHOLOGY

The agent of EBA establishes a chronic fetal infection with widespread gross and histologic lesions. The lesions develop progressively over a period of 3 months or more, with abortion typically occurring in the last trimester. As mentioned, the fetus is almost always fresh, fetal death having occurred from shortly before to shortly after delivery. Petechial hemorrhages are common in the mucosa of the conjunctiva and oral cavity and are especially distinctive along the ventral surface of the tongue (Fig. 52-2). Generalized enlargement of lymph nodes is usual. Some superficial nodes, such as the prescapular lymph nodes, can be readily palpated through the skin (Fig. 52-3). Whereas the prescapular lymph nodes of normal term fetuses weigh between 3.5 and 7.0 g, EBA fetuses may have prescapular lymph nodes that weigh 16 g or more.[10,11] Abdominal distention due to severe ascites is a variable feature, although some degree of ascites, often with strains of clotted fibrin, usually is present. An impressive but inconsistent gross lesion of EBA is an enlarged congested liver with a nodular capsule (Fig. 52-4). A more consistent gross change is splenic enlargement accompanied by enlargement of internal lymph nodes. The thymus may be reduced in size, with interlobular to diffuse hemorrhage and edema in the cervical portion. The lungs may be partially inflated. Other gross

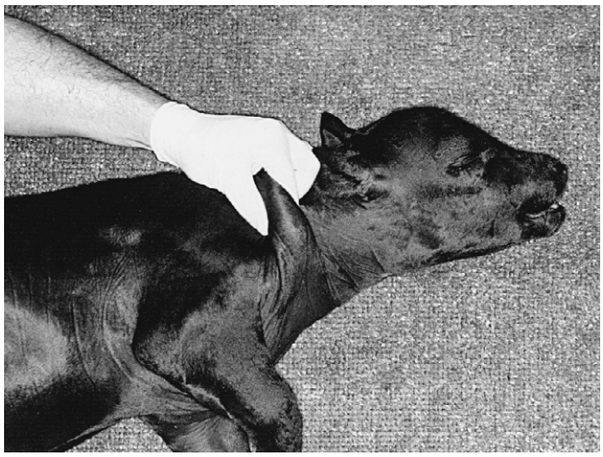

Fig. 52-3 Epizootic bovine abortion fetus, with an easily palpable, enlarged prescapular lymph node.

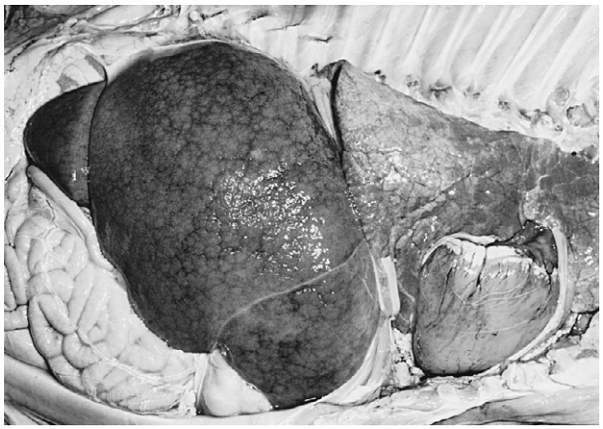

Fig. 52-4 Enlarged liver of an epizootic bovine abortion fetus, with a coarsely nodular capsular surface.

findings may include small gray inflammatory foci in the heart, kidney, or other organs.

Histologic examination of fetal tissues, particularly the lymphoid organs, is required to confirm a diagnosis of EBA.[10,11] The thymic lesions that develop late in the course of the disease apparently are unique to EBA (Fig. 52-5, *middle panel*), although not always present. Other features include loss of cortical thymocytes and infiltration of the medullary region with macrophages. Frequently, the interlobular septum is distended with fibrin and hemorrhage and is infiltrated by macrophages. The grossly evident enlargement of the lymph nodes is caused by lymphoid follicular hyperplasia with widespread macrophage infiltration in the sinuses and medulla. Lymphoid hyperplasia with macrophage infiltration also is present in the splenic white pulp. Late in the course of the disease, after the proliferative response, a common finding is acute necrosis in lymphoid organs, which may trigger the abortion. Also seen are widespread inflammatory lesions, which tend to have a vascular orientation in most organs, including the brain, the lungs, and the liver.

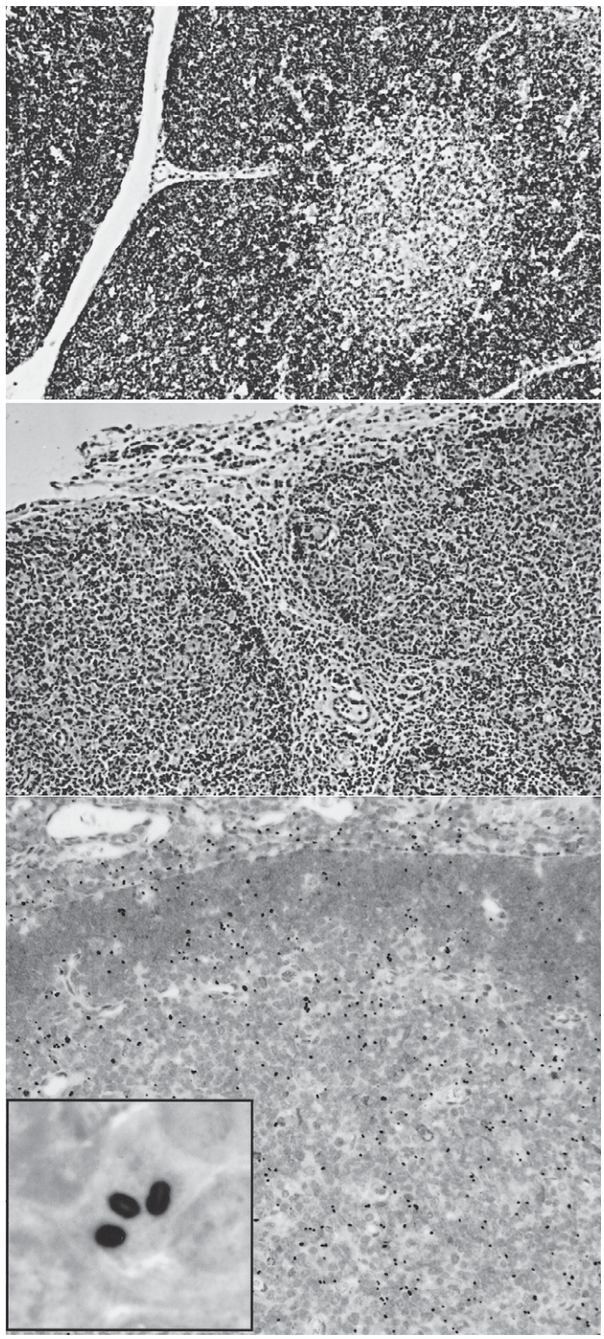

Fig. 52-5 Three photomicrographs of thymus from a normal *(top panel)* and two epizootic bovine abortion (EBA) fetuses *(middle and bottom panels).* In the normal fetus, a dense cortical mantle of thymocytes surrounds the medullary region. The interlobular septa are narrow and sparsely populated. In the EBA-affected thymus, the hematoxylin-eosin stain *(middle panel)* reveals thymic lobules that are reduced in size with loss of cortical thymocytes and macrophage infiltration. The interlobular septa are expanded by mixed cellular infiltrate. Immunohistochemical staining of an EBA thymus, using serum from an EBA fetus with high levels of immunoglobulin *(bottom panel),* reveals numerous short bacterial rods in the medullary zone, in the narrow cortical mantle, and in the septa. *Inset,* High-magnification view (oil immersion, approximately 1000×) of three bacterial rods within a cell in the medullary region.

In the brain, abnormalities include granulomatous meningitis with focal gliosis and vasculitis. The alveolar septa of the lung are thickened, with mononuclear cell infiltration and focal granulomatous infiltration in the interlobular septa. A consistent although less specific feature is granulomatous portal hepatitis with variable centrilobular congestion and hepatic cord atrophy.

DIAGNOSIS

The diagnosis of EBA is based on compatible pathologic findings and herd history with the elimination of other likely infectious causes. In some situations, however, when fetuses are extensively autolyzed and/or usable samples are limited, additional diagnostic procedures can be employed to demonstrate the bacterial agent associated with this disease. Modified Steiner silver stains may reveal small intracytoplasmic bacterial rods in the lymphoid tissues. Furthermore, an immunohistochemistry stain using serum from affected fetuses with elevated immunoglobulin levels provides improved identification of the bacteria (see Fig. 52-5, *bottom panel*).[6] The bacterial rods are widely disseminated in the lymphoid tissues. Although not yet being routinely applied to diagnosis, an EBA-specific PCR assay has been developed and validated for identification of the etiologic agent in both fetal necropsy tissues and the salivary gland of the tick vector.[5]

TREATMENT AND PREVENTION

Because the etiologic agent of EBA is not definitively identified and cannot yet be propagated in vitro, neither treatment nor vaccine is currently available. Rather, the cattle industry currently "manages around" EBA by manipulating the exposure of susceptible females. In areas in which the vector season is well defined and relatively limited (e.g., in the tick's northern ranges), some success has been achieved by those ranchers who have changed to fall calving. This tactic delays tick exposure of the pregnant cows until much later in gestation (e.g., at 7 months or later), with the result that exposure apparently occurs too late to destroy the fetus. Theoretically, such fetuses should mount an effective immune response to the agents of EBA. The apparent success of this "fall calving" strategy has lent strength to the hypothesis that fetuses must be exposed to the agents of EBA before the sixth month of gestation to manifest actual abortion.

In areas in which the vector season is prolonged (e.g., in the southern ranges of the tick), less benefit is derived from changing the calving season. That is, it is difficult to find a "window" of time during which animals can reach an advanced stage of gestation without also being exposed to ticks. Preventive efforts in these locales have involved the introduction of replacement heifers to endemic areas before their first breeding season, in order to induce immunity.

The apparent antibiotic susceptibility of the putative agent makes it tempting to recommend antimicrobial therapy to heifers at the time of or before exposure. Exposure can occur over such an excessive interval, however, that any treatment would have to be administered over a very long term. No recent data exist to support such an approach, although older anecdotal information suggested that feeding chlortetracycline during the tick season imparted some benefit. Well-controlled clinical trials are needed before such a strategy can be recommended.

Currently, researchers are making vigorous efforts to isolate and/or purify the causative agent from the fetal thymus in an attempt to develop a specific immunogen, and perhaps to formulate a rational treatment strategy.

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CHAPTER 53

Mycotic Bovine Abortion

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ETIOLOGIC AGENTS

Various molds and yeasts have been implicated in mycotic bovine abortion. Most of these agents are saprophytic fungi found in moist organic environments such as soil, hay, and poor-quality silage.

Aspergillus fumigatus is the most commonly isolated fungus, accounting for 60% to 80% of mycotic abortions.[1] Other *Aspergillus* species, including *Aspergillus flavus, Aspergillus terreus,* and *Aspergillus nidulans,* are encountered less frequently. The Zygomycetes compose the second most commonly encountered group; these include *Absidia corymbifera, Absidia ramosa, Mortierella wolfii, Rhizomucor pusillus,* and *Rhizopus arrhizus*.[1] *Pseudallescheria boydii* is another prevalent agent, albeit less commonly encountered. Species of *Penicillium;* dematiaceous fungi such as *Curvularia geniculata, Exophiala jeanselmei,* and *Wangiella dermatitidis;* and yeasts, usually of the genus *Candida* or *Torulopsis,* account for most of the remaining mycotic organisms encountered.[1] The relative frequency with which different fungi occur varies according to the geographic region. In northern New Zealand, for example, *M. wolfii* rather than *A. fumigatus* is the most common cause of mycotic abortion.[2]

EPIDEMIOLOGY

The incidence of bovine mycotic abortion varies widely, ranging from 2% to 20%.[3,4] Some surveys found mycotic agents to be the most commonly diagnosed cause of bovine abortion.[5] Mycotic abortions occur sporadically. Rarely are more than 10% of pregnant cows affected in a herd.[6] The age of the cow does not appear to be a factor predisposing to mycotic abortion.[7]

In the Northern Hemisphere, most mycotic abortions occur in the winter and spring months. Increased confinement of cattle during this period and feeding of hay or poor-quality silage are risk factors.[4] Confined housing in conjunction with feeding hay poses the greatest risk.[4] Some evidence suggests that abortions associated with *M. wolfii* are linked to nonstandard methods of silage preparation, particularly inadequate wilt-time before storage or inadequate silage compaction or storage due to incomplete sealing of the container.[5]

The relationship between frequency and amount of rainfall during the previous hay or silage-making season and the annual incidence of mycotic abortion remains unresolved. Some studies demonstrate a positive correlation; others have found no significant relationship.[4,8,9]

The suggestion that mycotic abortion is associated with the use of artificial insemination has not been substantiated.[5]

CLINICAL FINDINGS

Generally, mycotic abortions occur late in pregnancy, most often between 6 and 8 months of gestation, although they can occur as early as 2 months of gestation. Prodromal signs usually are not noted. The fetus is expelled soon after death in abortions caused by *Aspergillus* spp; however, it may be retained for up to 24 hours when a zygomycetous fungus is the etiologic agent.[1] The placenta frequently is retained and remains firmly attached. A secondary infection also may ensue.[10] Maternal caruncles may rupture at the peduncle and be expelled still attached to the cotyledons. This is most often observed when abortions are caused by zygomycetes.[6] Unless severe endometrial damage occurs, most cows recover sufficiently to have normal subsequent pregnancies.[4,10]

In approximately 25% of cows with *M. wolfii*–associated abortions, a fulminating, postabortion pneumonia occurs.[1] Cows exhibit rapid, shallow respiration with forced expiration.[11] Death usually occurs within 72 hours of onset of clinical signs.[2] Infrequently, signs of pneumonia are observed before the abortion.

PATHOGENESIS

The portal of entry of fungal agents is unknown; however, the respiratory and gastrointestinal tracts are the most likely routes of exposure. Although most fungal conidia that reach the lower respiratory tract remain there or are eliminated, it is speculated that some may enter blood vessels in alveolar septa and reach the placenta through the systemic circulation. Rumen infections, omasal ulcers, and intestinal lesions also may be factors predisposing to hematogenous spread to the gravid uterus, perhaps by facilitating penetration of mucosal barriers.[10] The hematogenous route is believed to be the primary route of infection because the lesion develops initially in the placentomes.[10] Once established, the infection spreads from the initial focus to the arcade zone of the placentome and advances laterally. Infection subsequently spreads to the interplacental space between cotyledons. Fetal tissues may be involved, primarily skin and lungs. Brain or liver involvement occurs occasionally.[10]

Mycotic abortions have been induced experimentally by intravenous administration of fungal conidia; however, intratracheal and oral administration have not successfully reproduced disease. Ascending infections in the genital tract are not thought to be a common route for infection. Attempts to experimentally induce abortion

with intrauterine inoculation of *Aspergillus* conidia have been unsuccessful.[12]

The pneumonia that develops in cows after *M. wolfii*–associated abortions is thought to result from a lung-uterus-lung cycle. Although the initial site of entry is unknown, spores most likely enter through the alimentary tract and pass by the venous or lymphatic system through the pulmonary vasculature, with subsequent localization in caruncles of the placenta. After abortion, a large number of fungal elements are absorbed from the uterus, causing an acute, fulminating, embolic pneumonia in the cow. This typically occurs 2 to 4 days after abortion.[13]

DIAGNOSIS

Samples for Examination

The placenta is the most useful sample for accurate diagnosis of mycotic abortion. If possible, the entire placenta should be submitted for examination, because infection may be restricted to only a portion of the placenta. Cotyledons with uterine caruncular tissue are the specimens of choice. The fetus or fetal tissues, particularly skin, lungs, and abomasal contents, also are useful diagnostic samples. Because the fetus is not involved consistently, however, submitting only the fetus or fetal tissues may lead to a false negative diagnosis. In *M. wolfii*–infected fetuses, liver and brain are the most commonly involved tissues.[13]

Gross Examination

Severe placentitis with necrosis of the cotyledons and leathery thickening of the intercotyledonary space is the most consistent finding in mycotic abortion. The cotyledons may have a coffee-bean or cup-shaped appearance due to the thickened margins of the cotyledons surrounding the attached necrotic caruncular tissue. Although the gross lesions in the placenta in mycotic abortions are quite distinctive, the lesions of other infectious causes of abortion, such as brucellosis and campylobacteriosis, can have a similar appearance.[6]

In approximately 25% of cases, the fetal skin has lesions. The lesions appear as raised, circumscribed plaques with a tendency to coalesce, somewhat resembling ringworm lesions. Areas around the eyes and on the head, shoulders, back, and sides are preferentially affected sites.[10] The skin lesions appear dry and wrinkled in infections with *Aspergillus* spp. but have a moist appearance when Zygomycetes are the inciting agents. The fetus may appear emaciated and dehydrated, exhibiting an overall wrinkled appearance to the skin.

Direct Microscopic Examination

Scrapings from placenta and affected skin, as well as abomasal contents, should be examined for fungal hyphae after the samples are digested with a 10% potassium hydroxide solution. Most organic substances are cleared with this treatment; however, fungal hyphae are unaffected and are readily visualized microscopically. Methods using calcofluor white or chlorazol black E also can be used to enhance visualization of fungal hyphae.

Finding hyphae in placental or fetal skin scrapings in conjunction with compatible gross lesions provides a rapid presumptive diagnosis of mycotic abortion. The presence of fungal hyphae in the abomasal fluid correlates well with mycotic abortions; occasionally, however, fungal hyphae are found in the abomasal fluid as an incidental finding.

The microscopic appearance of the hyphae can be a clue to the particular fungal agent involved. *Aspergillus* hyphae are 3 to 6 μm in diameter, with parallel sides and frequent septation; typically, hyphae branch in a dichotomous pattern. The hyphae of *P. boydii* are similar to those of *Aspergillus* organisms except that the branching pattern is more irregular. The hyphae of the Zygomycetes are broad (5 to 20 μm), thin-walled, and pleomorphic. The hyphae tend to branch irregularly, sometimes at right angles, and are nonseptate, although occasional septa are detected.[14] It is not possible to distinguish among genera of the Zygomycetes on the basis of hyphae in tissue digestions or histologic sections. The presence of budding yeasts and pseudomycelia or brown-pigmented hyphae may suggest an infection caused by a yeast or one of the dematiaceous fungi, respectively.

Histopathologic Examination

The placenta is the most severely affected tissue in mycotic abortions. Typically, wedge-shaped areas of necrosis or hemorrhage, or both, occur in the caruncle as a result of necrotizing vasculitis and thrombosis. Cotyledonary villi and placental stroma often are secondarily involved by local or hematogenous spread. Fungal hyphae can be found in vascular structures, thrombi, necrotic tissue, and adjacent viable tissue. The zygomycetous fungi are more easily visualized than *Aspergillus* organisms in hematoxylin-eosin–stained tissue sections. In general, fungal elements are best visualized in tissue using the Gridley fungus stain or Gomori's methenamine silver nitrate stain or by the periodic acid–Schiff reaction. Microscopic fungal morphologic characteristics, as previously described, can be used to suggest the group of agents involved.

The skin lesions of mycotic abortions involve predominantly the epidermis and dermis, with a parakeratosis, necrosis, and neutrophilic infiltration. In some instances, dermal vessels are thrombosed, and fungal elements can be seen invading the dermis. When the lungs are involved, a purulent bronchopneumonia with hyphae in the bronchioles can be observed. Focal hepatic necrosis can occur and is a relatively common finding with *M. wolfii*–associated abortions.[13] Occasionally, encephalitis with perivascular cuffing, neutrophilic infiltration, and necrosis of the neuropil is observed.

Mycologic Examination

Culture results alone should be interpreted with caution. Because the placenta may be contaminated, the morphologic characteristics of fungi recovered should be consistent with the morphologic appearance of fungal

hyphae observed by direct, microscopic, or histopathologic examination before the fungus is designated as the etiologic agent. Cultures from the abomasal contents are less prone to contamination.

For isolation, fungal media with antibiotics should be used to suppress bacterial contamination. Sabouraud's dextrose agar and potato-dextrose agar are commonly used media. Cultures are routinely incubated at 25° to 30°C for up to 4 weeks.

Isolation of zygomycetous fungi can be difficult.[2] They do not remain viable in tissues as long as other fungi do. Toxic effects from tissue autolysis are thought to be responsible, in part, for the failure to recover organisms when they are observed in tissue sections.

Molds isolated from the placental or fetal tissues are identified on the basis of colonial and microscopic morphology. Speciation can be difficult, especially with the zygomycetous fungi, and may require mating studies, which is beyond the capability of most clinical laboratories.

Yeasts are most easily identified with the use of commercially available assimilation systems.

MANAGEMENT AND TREATMENT

Because of the sporadic nature of mycotic abortions and differing management and feeding practices, specific control efforts should be tailored to the scenario at hand. When warranted, changes in housing to decrease confinement and cow density, as well as improving ventilation, will limit exposure to fungal conidia. Limiting the feeding of moldy hay or poor-quality silage, particularly to pregnant animals, is important in minimizing the potential for abortion.

Specific antimycotic treatments for cows that abort usually are not required. Instead, efforts should be directed at minimizing endometrial damage resulting from retained placenta or subsequent secondary infection.

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CHAPTER 54
Reproductive Toxicants

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Reproductive toxicology is the study of toxicant exposures that adversely affect sexual behavior, gametogenesis, conception, development, parturition, or healthy maturation of progeny. Even under normal physiologic conditions, reproductive function is subject to failure. It is therefore not surprising that exposure of this system to toxic insult interferes with the complex biologic processes that culminate in healthy offspring. The true incidence of toxin-induced reproductive dysfunction is unknown but presumably is well below that due to infectious and management-related causes.

The incidence of malformed calves has historically been underreported because of the negative impact on the sale of breeding stock, or malformations have been undiagnosed because the death of offspring was not associated with easily identifiable anomalies.[1] Experimental evidence, however, confirms that poisonous plants can induce livestock losses as a result of embryonic death, abortion, and teratogenesis. The relationship between exposure and reproductive dysfunction is complex because exposure of the dam, the sire, or both may influence reproductive outcome. In addition, exposures may have occurred in the distant past, immediately before conception, or during gestation. The timing of toxin exposure before or during gestation can be identified for some specific dysfunction, and for others it cannot. Examples include chromosomal abnormalities detected in the embryo that arise from mutations in the germ cells of either parent before conception, or from direct exposure of the embryo/fetus during gestation. Malformations usually occur with exposure during a discrete period of gestation, extending from about day 40 to day 90 of bovine fetal development.

POISONOUS PLANTS

Bovine reproductive toxins are derived primarily from plants. The importance of the contribution made by poisonous plants is best appreciated by considering the pervasive exposure of cattle on rangelands and pastures. Experimental evidence has substantiated the reproductive toxicity of a number of range and cultivated plants.

Poison Hemlock

Poison hemlock is most likely to be ingested in the spring, because it often is one of the first green forages to appear. Ingestion of poison hemlock *(Conium maculatum)* by pregnant cows during the gestation interval of days 50 to 75 induces multiple congenital contractures (MCCs) in calves, a condition commonly referred to as "crooked calf disease."[2] Pregnant cows gavaged with the fresh green plant during days 50 to 75 of gestation had calves with arthrogryposis and spinal curvature, defects similar to those induced by administration of the isolated teratogenic principle coniine. Dams gavaged with the dry plant had either normal or equivocally deformed offspring, whereas those fed 410 to 840 g of fresh green plant daily from day 50 to 75 of gestation either aborted or delivered calves with limb deformities. Periodic ultrasound scanning during the treatment period revealed severe inhibition of fetal movement in goats.[3] Suppression of fetal movement during the critical gestational window is thought to be the cause of the plant-induced cleft palate (suppression of tongue thrusting) and skeletal contractures. A similar mechanism is likely in cattle.

Lupines

Certain species of the *Lupinus* genera cause MCCs in calves exposed in utero during days 40 to 70 of gestation.[4] Certain *Lupinus* species, such as *Lupinus sericeus* or *Lupinus caudatus,* contain anagyrine as the principal alkaloid and are teratogenic in cattle, but not in sheep and goats. The teratogen in *Lupinus formosus* is thought to be ammodendrine.[5] This plant species has induced severe limb and spinal deformities together with cleft palate in calves. These defects were induced in calves when dams were dosed with 2.33 to 3.16 g of fresh plant/kg of body weight twice daily during days 40 to 70 of gestation. More recent data from epidemiologic studies suggest that the gestational period of teratogenic susceptibility may extend to day 100.[6] The MCCs are similar to those induced by *Conium* and *Nicotiana* genera and include arthrogryposis, torticollis, kyphosis, scoliosis, rib cage deformities, and extension or flexure of the carpal or tarsal joints.

Tobacco

Nicotiana glauca (tree tobacco) is a small tree commonly found at lower elevations of California and Arizona. Seven cows dosed with dried, ground *N. glauca* daily from the 50th to 75th day of gestation produced seven calves with arthrogryposis of the forelimbs and curvature of the spine.[7] Other abnormalities included general malpositioning and misalignment of the distal ends of the radius and ulna and the proximal ends of the metacarpal bones. Carpal joints were severely affected, fetlock and pastern joints less so, with lateral rotation of forelimbs common.

One of four calves had moderate torticollis and scoliosis. Anabasine, not nicotine, is thought to be the teratogen. *Nicotiana tabacum* (common tobacco) is a known teratogen in swine,[8] but such effects have not been documented in cattle.

Locoweeds

From an economic standpoint, the locoweeds—certain *Astragalus* and *Oxytropis* species—have more of an adverse impact on bovine reproduction than any other group of poisonous plants. Of the almost 400 species of *Astragalus* and 22 of *Oxytropis,* fewer than 20 have been verified to cause true locoism and the associated reproductive dysfunction. Undoubtedly, many more species will be found to contain swainsonine, the active toxic principle in locoweed. The multiple reproductive problems induced by chronic locoweed consumption include abortion, teratogenesis, fetal death, delayed placentation, deficient uterine and placental vascular development, hydrops amnii, hydrops allantois, abnormal cotyledonary development, decreased conception rate, reduced libido, and diminished sperm production.[9] Enlarged ovaries with extensive follicular-type development have been observed in prepubertal calves intoxicated with locoweed.[10] Cytoplasmic vacuolation is seen in luteal cells and in atretic follicles, which may affect normal progesterone and estrogen levels.[11] Cytoplasmic vacuolation of the pituitary may affect normal gonadotropin levels in both sexes, thereby influencing reproductive performance.[12]

Locoweed poisoning is a chronic condition requiring continuous ingestion for 4 to 6 weeks. Initially, cattle may be reluctant to graze locoweed, but once started, they become habituated, often seeking it out while excluding appropriate forage from their diet. Recovery is possible if cattle are removed from infested ranges and supplied with suitable feed before they become too emaciated. Some *Astragalus* locoweeds are perennials that thrive only during favorable environmental conditions.[13] Flourishing and declining locoweed populations on western ranges are associated with the waxing and waning of reproductive and other acute and chronic disorders in indigenous cattle. *Oxytropis* locoweeds cause more consistent annual losses, coincident with more stable plant populations.

The toxic principle of locoweeds and the *Swainsona* genus of Australia is the indolizidine alkaloid swainsonine. Swainsonine interferes with oligosaccharide degradation and glycoprotein processing by inhibiting lysosomal α-mannosidase and mannosidase II. Resulting lesions are characterized microscopically by cytoplasmic vacuolation of cells in a variety of tissues.[14] Because locoweed poisoning is an induced storage disease, postmitotic cells such as neurons and cardiac myocytes are most vulnerable. Involvement of these cells leads to clinical signs of central nervous system derangement (locoism) and heart failure. Vacuolar degeneration also is observed in other tissues, including the gonads, thyroid gland, pancreas, lymph nodes, and placenta. Lesions in the fetus are similar to those in the dam, presumably because swainsonine traverses the placental barrier.

In pregnant animals, both dam and fetus are affected, with abortion and teratogenic effects recorded. Locoweeds are unique in possessing a broad gestational window for the induction of toxic effects on the fetus. Abortion, the most common untoward effect, can occur at any stage of gestation after ingestion of locoweed for several weeks. Two cows dosed with *Astragalus lentiginosus* through a rumen fistula at 0.6 kg for the first 120 days of gestation aborted deformed fetuses by day 173.[15] Skeletal malformations commonly include limb and joint abnormalities. The fetus normally is hypoxic and hypertensive relative to the dam because of fluid-filled lungs, with the fetus receiving oxygen from the placenta. Locoweed is thought to induce increased vascular resistance in the fetus, an effect similar to that in cattle at high elevations.[1] The increased workload on the fetal heart results in hypertrophy, dilatation, cardiac insufficiency, fluid accumulation, fetal death, and subsequent abortion. In ewes fed *A. lentiginosus,* serum progesterone and cotyledonary prostaglandin concentrations were altered[16]; presumably, cattle are affected in similar fashion. A dose-dependent reduction in serum progesterone concentration and a significant increase in cotyledonary prostaglandins were observed.

Real-time ultrasound imaging was used in the pregnant ewe model to demonstrate the adverse effects of locoweed on fetal growth and development.[17] Ewes fed locoweed during gestational days 60 to 100 developed irregularly shaped cotyledons, with many of these structures becoming atretic. Excessive fluid accumulated in the placenta, leading to fetal heart arrhythmias. During dosing from days 60 to 70 of gestation, the heart rate in affected fetuses initially exceeded 200 beats per minute. With continued exposure to locoweed, fetal heart rate slowed to 60 beats per minute, and 3 days later fetal cardiac arrest occurred, followed by abortion in 40 to 72 hours. Fetal heart arrhythmias occurred concomitantly with cotyledon atrophy and hydrops allantois. Necropsy revealed fetal cardiomegaly. Similar fetal effects are thought to occur in cattle.

Effects of locoweed on the male reproductive tract have been studied in the ram; however, the effects can be extrapolated to other ruminant species. Spermatogenesis was decreased, and numerous cytoplasmic vacuoles have been reported in Sertoli cells, spermatogonia, and primary and secondary spermatocytes.[18] Epithelium lining the epididymis, vas deferens, and seminal vesicles was severely hypertrophied and vacuolated. The lumen of the epididymis was practically devoid of sperm. Glandular tissue of the bulbourethral glands was mildly vacuolated.

Pine Needle Abortion

Needles from the Ponderosa pine tree *(Pinus ponderosa),* lodgepole pine *(P. contorta),* and common juniper *(Juniperus communis)* cause abortion primarily when consumed during the last trimester.[19] Consumption by pregnant cows induces a premature parturition or abortion usually in 1 to 3 days but may be delayed for 2 to 3 weeks.[20] The greatest response from ingestion of pine needles occurs when ingested for a period of 3 days or more

and at a relatively high level (>2 kg/day). Severe winter weather often leads to conditions in which cattle mingle under trees to escape wind and snow. Some calves from affected dams are born weak but may survive with adequate care. Common sequelae include retained fetal membranes and metritis. A study examining the effects of dietary variables on consumption of pine needles and parturition concluded that (1) feeding high levels of protein increased pine needle consumption but not abortion rate, (2) weathered or aged pine needles had activity equivalent to or greater than that of fresh needles, and (3) feeding corn silage to cows prevented pine needle consumption.

Isocupressic acid (ICA) is the abortifacient principle in Ponderosa and lodgepole pines, as well as in common juniper.[19] It is contained in green or dry needles, bark, and branch tips. Isocupressic acid, like acetyl-ICA and succinyl-ICA, is classified as a labdane resin acid. All of these resin acids have induced abortions in cattle. The rumen microbes rapidly hydrolyze acetyl- and succinyl-ICA to ICA, the direct abortifacient. Isocupressic acid levels were 0.8% and 2.0% (dry weight) in lodgepole pine and common juniper, respectively.

The mechanism of pine needle abortion involves a profound constriction of the caruncular arterial bed.[21] Furthermore, pine needle extracts and plasma from fed cows increased uterine arterial tone in vitro. After consumption of pine needles by beef cows in late gestation, uterine arterial blood flow progressively decreased to less than 50% of prefeeding rates before premature delivery of live weak calves. In another study, uterine blood flow in cows fed pine needles decreased progressively, declining to 25.5% of baseline by the day of premature parturition.[22] The evidence suggests that consumption of pine needles induces a progressive reduction in uterine blood flow to the gravid horn and that this reduction causes the onset of premature parturition accompanied by normal prepartal changes in steroid secretion.

Broomweed

Perennial broomweeds or snakeweeds (*Gutierrezia microcephala* or *Gutierrezia sarothrae*) infest vast expanses of arid western rangelands. Although unpalatable, broomweed is one of the first green plants to emerge in the spring. Broomweed causes abortion or premature birth of weak calves. In affected herds, 10% to 60% of calves typically are involved. Of 52 cows fed *G. microcephala,* 23 cows delivered normal calves and 27 cows delivered premature, weak, or dead calves weighing 7.5 to 50 pounds. Broad gestational susceptibility of the fetus to broomweed is apparent from these early experiments. Thirty-four cows had placentas retained for 3 to 10 days after calving. Several cows had signs of systemic illness, and four either died or were euthanized. The toxicity of the broomweed varies according to growing conditions from year to year, stage of growth, and soil type.[23] Broomweed grown on sandy soil is more toxic than that grown on hard soils. The toxic principles in broomweed include mono- and diterpenes, saponins, oxygenated flavonol methyl esters, and yet-to-be-characterized saponins.[24] Specific abortifacients have not been identified.

Fescue

Fescue *(Festuca arundinacea)* toxicosis is caused by ingestion of fungus-infected *(Neotyphodium coenophialum)* tall fescue, mostly the Kentucky-31 cultivar. *N. coenophialum* is a seed-borne, intercellular, systemic fungus that resides in the leaf, sheaths, and flower of the grass host.[25] With 35 million (mostly fungus-infected) acres in the eastern and midwestern United States, the impact on bovine performance is enormous. Newer varieties of fescue have been developed that are free of the endophyte fungus. Unfortunately, the hardiness of this cool-season grass is considerably diminished in the absence of the fungus. Clearly, although it is toxic to cattle and other livestock, the fungus confers stress tolerance to the grass. Fungus-free fescue is less tolerant to weather-related and other stressors, including overgrazing. Cattle seem to like it more, but so do insects, nematodes, and plant pathogens.

The most critical consequence of feeding infected fescue in cow-calf operations is diminished reproductive efficiency. This condition usually is associated with a syndrome commonly referred to as "summer slump" and is pronounced when ambient temperature exceeds 32°C (89°F).[26] Toxic fescue forage fed to steers during the winter months did not cause health problems or reduction in performance; however, it did depress circulating PRL in a dose-dependent manner.[27] Clinical signs of summer fescue toxicosis are exacerbated by elevated environmental temperatures because of a decreased ability to dissipate excess body heat.[26] This is due largely to the peripheral vasoconstrictive effects of the toxic ergot peptide alkaloids (primarily ergovaline) produced by the endophyte fungus *N. coenophialum.* Adverse effects were recorded in two groups of cattle exposed to fescue containing 285 and 381 parts per billion (ppb), respectively, of ergovaline. The vasoconstrictive effects of another endophyte-produced compound, *N*-acetylloline, on the bovine lateral saphenous vein may contribute to fescue-associated problems.[28] These studies suggest possible additive effects of *N*-acetylloline and ergovaline and support the concept that a redistribution of blood flow to internal organs somehow compromises reproduction. Other signs associated with the summer toxicosis syndrome in general include slobbering, open-mouth breathing, reduced average daily gain, and a propensity to spend daytime hours in water or shade, rather than grazing. Fescue-induced hyperthermia also appears to be partly responsible for the decreased intensity of estrus and an increased embryolethality. This is consistent with the finding that bovine embryos incubated at 40°C for 48 to 60 hours suffer a dramatic increase in mortality.[29]

Reduced calving rates are attributed to feeding endophyte-infected fescue.[30] Ninety-six percent of beef heifers raised on low-endophyte fescue (0% to 5% plants infected) conceived, compared with 55% of those raised on high-endophyte fescue (80% to 90% plants infected). Of primiparous cows grazing highly infected pastures, 33% conceived, versus 93% on low-endophyte pastures. Conception rates decreased 3.5% for each 10% increase in fungal infection. In a 3-year study, 39% of animals on

high-endophyte fescue raised a calf, versus 65% of those on low-endophyte fescue pasture.[31] The proportion of heifers with surviving calves was 11% with high-endophyte fescue versus 58% with low-endophyte fescue in the first-year, 63% versus 84% in the second year, and 42% versus 53% in the third year. First-service artificial insemination (AI) conception rates for the high-endophyte–exposed heifers were reduced for the first 2 years of the study (45% versus 74%)[32]; however, the overall first-service conception rates among those cows inseminated for the 3-year period were 74% and 78% for the high- and low-endophyte groups, respectively.

Alterations in the hormonal milieu of cattle also are associated with infected fescue ingestion. Circulating prolactin (PRL) levels are depressed in cattle consuming infected fescue.[33] Dopamine is a known inhibitor of PRL secretion, and elevated circulating dopamine is associated with decreased serum PRL.[34] The dopamine-like activity of a toxic fescue diet is reflected by the significant depression in serum PRL within 48 hours after ingestion commences.[26] This biomarker of exposure is sustained regardless of ambient temperature effects, indicating that certain hormone levels are altered even in the absence of high environmental temperatures. Heat stress does appear to potentiate the adverse effects of infected fescue on reproductive function, however. McKenzie and Erickson[35] reported a 23% reduction in basal luteinizing hormone (LH) concentrations in heifers fed fescue hay. Other investigators, however, found no effect of endophyte-infected fescue on serum LH despite ergovaline intake at least 50% greater than the typical field exposure.[36] Altered luteal function was reported in 62% of heifers in which a corpus luteum was detected by ultrasonography.[37] Serum progesterone level was depressed and was unrelated to decreased average daily gain; however, it was prevented by high-energy supplementation. Puberty, as detected by the first sustained increase in serum progesterone, was delayed in Angus heifers raised on endophyte-infected fescue.[31]

These reports substantiate the adverse effects of toxic fescue on bovine reproductive function. Treatment of intoxicated cattle is neither practical nor economically feasible, and it is not particularly efficacious. Several mineral supplements have been touted as alleviating the clinical signs of fescue toxicosis. To date, little scientific evidence is available to support their efficacy. Slow-release formulations of thiabendazole[38] and ivermectin[39] obviate some of the adverse effects of the endophyte toxins. Thiabendazole has been shown to reduce some of the vasoconstrictive activity of toxic fescue. Enhanced reproductive performance, however, has not been demonstrated. Internal parasites do reduce forage intake in cattle, which translates into a reduction in gain. Work done by Dr. George Garner at the University of Missouri with cattle in environmental chambers indicates that parasites tend to lower the temperature threshold at which animals show signs of fescue toxicosis. The decreased sensitivity to summer fescue toxicosis, together with increased intake, may partially explain why dewormers improve performance. Metoclopramide, a dopamine receptor antagonist, was shown to increase the concentration of serum PRL, prolong grazing time, and maximize daily gain in steers ingesting toxic fescue.[40] Further research in these areas is necessary to demonstrate an effective cost-benefit ratio.

Toxic fescue pastures can be either renovated or managed; the decision is an economic one. To remove endophyte-infected fescue, the application of an effective herbicide is followed by a smother crop such as wheat or sudan grass, harvesting of the smother crop, and another application of herbicide if necessary.[41] Cattle can be rotated off fescue during the hot summer months to increase gains. Toxic pastures also can be interseeded with legumes such as red clover, lespedeza, and birdsfoot trefoil for the dilution effect. Supplementation with corn at approximately 0.6% of body weight will allow efficient forage digestion and reduce the toxic effects of fescue. Avoiding the excessive application of nitrogen fertilizer reduces the concentration of ergovaline in toxic fescue. Application and type of fertilizer can be selected to encourage the growth of legumes. Cattle should not be permitted to graze the most toxic portion of infected fescue, the seedheads. Clipping or heavy grazing will prevent this from occurring. Finally, the ammoniation of toxic fescue hay is highly recommended to increase digestibility, reduce the toxic effects of ergovaline, and increase daily gains by 50% or more.

Gossypol

Gossypol is the primary toxic principle found in whole cottonseed and cottonseed meal. It is a yellow, polyphenolic pigment produced in high concentrations in the seeds of the cotton plant (genus *Gossypium*). Being toxic, gossypol imparts insect resistance to cotton, making low-gossypol varieties subject to considerable damage. In a different way, gossypol has a cumulative toxic effect in monogastric and ruminant animals. During processing the seeds are sliced into flakes to facilitate extraction of oil for use in salad oil, margarine, and shortening. The remaining cottonseed flakes, together with some free gossypol, are ground into cottonseed meal for use as a protein supplement for livestock.[42] Gossypol poisoning rarely occurs and usually can be traced to situations in which gossypol concentrations in the ration were unknown or recommended safe levels were exceeded.

Gossypol content of whole cottonseed and cottonseed meal varies according to the species of cotton, environmental conditions during the growing season, and the method of oil extraction.[42] It exists in two forms: the free, toxicologically active form and the inactive, protein-bound form. Gossypol in unprocessed seed exists exclusively in the toxic unbound form and is readily absorbed from the gut of monogastrics. Mature ruminants, however, are relatively resistant to free gossypol because of their ability to detoxify it by binding to rumen proteins. High free gossypol intake can overwhelm this detoxifying mechanism, leading to obvious clinical intoxication[43] or insidious effects on reproduction.[44,45] Mature ruminants can tolerate large quantities of free gossypol without apparent adverse effects; however, reduced performance was detected in dairy cows fed rations containing 2250 parts per million (ppm) for several weeks.[46]

The adverse effects of gossypol on reproduction were first recognized in humans in China during the 1930s and 1940s.[47] Not a single childbirth was reported in an area of China where out of economic necessity, crude cottonseed oil was substituted for soybean oil in cooking. Afflicted women suffered from amenorrhea, and the men were impotent. Subsequent studies of gossypol as a birth control agent in humans received widespread attention.

To assess the adverse effects of gossypol on bovine reproduction, different levels of gossypol in rations have been studied. Brangus heifers fed diets containing either 5 g of free gossypol/day from direct solvent–extracted cottonseed meal or 15 g free gossypol/day from cottonseed for 70 days were evaluated, after follicle-stimulating hormone (FSH) treatment, for adverse effects on embryo development, grade, and viability[48] and follicular development and function.[49] The number of follicles and follicular size were reduced in heifers receiving 5 g of free gossypol/day from direct solvent cottonseed meal but not in heifers receiving 15 g of free gossypol from cottonseed. Embryo quality, developmental stage, and viability indexes were not significantly different for heifers fed the control diet and those fed the gossypol diets; however, a higher percentage of degenerative embryos were found in heifers receiving 5 g free gossypol from cottonseed meal than in those fed either of the other regimens. By contrast, another study in which direct solvent cottonseed meal and cottonseed were used to provide free gossypol intakes of 0, 0.4, 1.7, 3.3, and 8.2 g/day for 62 days to postpubertal beef heifers showed no treatment-related difference in 30-day pregnancy rates after removal of gossypol from the diet.[50] Heifers consuming 10 to 20 g/day of dietary free gossypol for 1 to 2 months exhibited increased red blood cell fragility but no detrimental effects on growth rates or reproduction.[51] Lactating cows fed 20 mg free gossypol/kg of body weight for 33 weeks did not have significant differences from control animals in preovulatory LH surge, luteal phase concentrations of progesterone, follicular fluid concentrations of estradiol and progesterone, and 60-day pregnancy rates.[51] Gossypol interferes with normal in vitro development of embryos and synthesis of progesterone by luteal cells.[52] These findings have not been corroborated in vivo. Current evidence indicates that rations containing reasonable concentrations of gossypol are unlikely to adversely affect reproductive performance in heifers and cows.

Gossypol impairs reproduction in laboratory animal and human males at concentrations sometimes encountered in commercial livestock rations.[44] In bovine males fed gossypol-containing rations, histologic evaluation revealed an increased seminiferous tubule lumen diameter, thinner germinal epithelium wall, and a decreased number of germ cell layers.[53,54] Spermatocytes were present in the lumen of seminiferous tubules of bulls fed gossypol but not in control animals. The cellular damage is confined mainly to the intraluminal cell layers. Brahman bulls fed a ration of cottonseed meal and corn in a formulation to deliver 8.2 g of free gossypol per bull per day had a lower percentage of normal spermatozoa compared with control animals (49% ± 9.8% versus 83% ± 3.2%) by week 5. The abnormality was restricted primarily to midpiece morphology.[55] Sperm motility also was depressed in gossypol-fed bulls by week 9. These studies reveal the relative insensitivity of ruminant females to dietary gossypol. By contrast, bulls show marked testicular damage, implying that free gossypol intake should be restricted.

Poisonous Plants of Lesser Importance

Consumption of *Iva angustifolia* (narrowleaf sumpweed) has been associated with mid- to late-gestation bovine abortion, lactogenesis, vaginal mucous discharge, and placental mineralization.[56] Similarly, *Cupressus macrocarpa* (cypress tree) ingestion has been associated with abortion in cattle and cerebral leukomalacia in the aborted fetus.[57]

PHYTOESTROGENS

Phytoestrogens are estrogen mimics produced in relatively large amounts by certain legumes. The quantity of phytoestrogens is rarely sufficient to cause total reproductive failure, but subclinical impairment is common in sheep.[58] Ingestion of excessive forage estrogens by ruminants can cause hyperestrogenism, with nymphomania, cystic ovaries, and swollen genitalia, or antiestrogenic effects of gonadal hypoplasia and anestrus, depending on the action of the compound on estrogenic receptors.[59]

The contribution of phytoestrogens to reproductive failure in cattle is largely anecdotal, with limited documentation by controlled studies. In a herd of 700 cows, the pregnancy rate dropped to 45% in cattle pastured on burr medic (*Medicago* spp.), known to contain the proestrogens formononetin and coumestrol.[60] In another herd of 100 cows, the pregnancy rate dropped to 10%, with shortened estrous cycles in cattle pastured on subterranean clover *(Trifolium),* which contains the proestrogens isoflavone biochanin A and genistein. The influence of phytoestrogens was studied in three ovariectomized heifers, each fed 20 kg of 100% red clover silage daily for 14 days.[61] Behavior, reproductive organs, and pituitary response to exogenous gonadotropin-releasing hormone (GnRH) injections were monitored. Clinical effects included development of edema and mucous discharge from the vulva, presence of milky fluid in the mammae, and increases in teat size and cross-sectional area of the uterus. The red clover silage diet appeared to attenuate the pituitary response to GnRH. The concentration of phytoestrogens in forage depends on the time of harvest and methods for preservation. Field-curing hay reduces estrogenic activity by approximately 70%, whereas ensiling preserves it.[62]

NITRATE-INDUCED ABORTION

Many species of animals are susceptible to nitrate (NO_3^-) poisoning, with cattle being the most frequently affected. Ruminants are especially vulnerable to nitrate intoxica-

tion because of the nitrate-reducing potential of rumen microbes. Cattle are therefore susceptible to both preformed nitrite, such as from a fertilizer source, and nitrate from nitrate-accumulating plants or fertilizer. The reduction of NO_3^- to the more toxic nitrite (NO_2^-) is an intermediate step in the bacterially mediated biochemical sequence of the formation of fully reduced ammonia (NH_3). The relatively toxic nitrite ion can be absorbed into the bloodstream, where it oxidizes normal ferrous iron (Fe^{2+}) in hemoglobin to the ferric state (Fe^{3+}), forming methemoglobin. Methemoglobin is unable to carry oxygen, resulting in hypoxemic stress in supplied tissues. Excessive maternal exposure to nitrate or nitrite may be a cause of late-gestation abortion in cattle. Forage nitrate concentrations of approximately 1% are considered potentially toxic to cattle. Field cases of bovine abortion have been associated with forage containing as little as 0.52% nitrate, and abortion usually is reported to occur with feeds containing 0.61% to 1.0% nitrate.[63] Common examples of nitrate-accumulating plants include Sudan grass, oat, wheat, corn, pigweed, and Johnson grass. Heavy fertilization associated with cool, cloudy growing conditions is conducive to nitrate accumulation.

Placental transfer of oxygen may be greatly reduced, with consequent hypoxia and intrauterine death of the fetus, in nitrate-poisoned cows. Further adverse effects of nitrite include its vasodilatory action and the consequent reduction in mean arterial blood pressure and oxygen tension.[64] Hypoxemia in late-gestation fetuses results in increased cortisol[65] and ACTH concentrations in fetal plasma.[66] Nitrite-induced fetal hypoxemia could result in abortion as a result of anoxic death of the fetus, as well as activation of the fetal adrenals. Sodium nitrite given intravenously to pregnant cows at a dosage of 20 mg/kg of body weight was fatal to two of eight fetuses within 24 hours.[67] Nitrate concentration in fetal ocular fluid of casarean-delivered fetuses equaled or exceeded the perinatal concentrations of 20 µg NO_3^-/ml (20 ppm) found in specimens diagnostic for excessive nitrate exposure.

MYCOTOXINS

Reproductive failure in livestock often is thought to be a feed-associated problem when an infectious cause cannot be diagnosed. Evidence to support such claims frequently hinges on the chemical identification of mycotoxins in representative feed samples. Unfortunately, few experimental studies provide definitive proof of the role of mycotoxins in bovine reproductive disease. Empirical and anecdotal evidence demonstrating a cause-and-effect role for mycotoxins involved mycotoxin concentrations rarely found in naturally contaminated rations.

The estrogenic mycotoxin **zearalenone** has a potent effect in female swine at concentrations of 1 to 2 ppm in complete rations. The relative resistance of cattle to zearalenone is illustrated by the following studies. Cycling, virgin Holstein heifers given daily oral doses of 250 mg zearalenone (equivalent to 62.5 ppm in the dairy ration fed these heifers) for one nonbreeding estrous cycle and the following two estrous cycles during which the heifers were artificially inseminated had a 62% conception rate, versus 87% in control animals.[68] Serum progesterone was unaffected. Doses up to 500 mg of 99% pure zearalenone given once per day to 18 nonpregnant, multiparous dairy cows for two consecutive estrous cycles had no effect on serum progesterone or estrous cycle length.[69] An estrogenic mycotoxicosis induced by consumption of moldy forage was observed in three Friesian cows and four buffaloes.[70] Similar changes were induced in mouse reproductive tracts when the animals were injected with the moldy hay extract.

Ergot alkaloids, produced by the *Claviceps* genus, have been incriminated in various reproductive diseases. *Claviceps* fungi invade the ovary of various grasses and small grains, replacing these structures with dense fungal masses referred to as **ergot bodies** or sclerotia. Ergot bodies are the source of physiologically active ergot alkaloids found in contaminated grains such as rye, barley, oat, wheat, fescue, and bromegrass. The ergotamine group of ergot alkaloids (i.e., ergopeptine alkaloids, which cause myometrial contraction in late pregnancy) induce a marked peripheral vasoconstriction, resulting in gangrene of the distal extremities.[71] The natural alkaloids of ergot also are potent stimulants of uterine muscle.[72] Small doses stimulate increased force and frequency of contraction. The sensitivity of the uterus to ergot alkaloids varies with maturity and the stage of gestation. Exposure of the gravid uterus during late gestation elicits forceful, prolonged contractions with a marked increase in tone. In sheep, supplementary feeding of a ration containing 0.1%, 0.5%, or 0.7% ergot bodies by weight decreased the number of ewes lambing by 20%[73]; by extrapolation, similar results could be expected in cattle. Abortion occurred in 11 of 36 cows in late gestation 7 days after being placed on a ryegrass pasture.[74] At least 25% of the seedheads of ryegrass contained ergot bodies. Aside from the adverse effects on reproduction, ergotism promotes agalactia in several livestock species including cattle.[75]

Bovine abortion has been associated with consumption of aflatoxin-contaminated peanuts.[76] Eleven of 14 cows in late gestation aborted after consumption of aflatoxin-tainted peanuts for 4 days. Eight of the cows died as a result of acute aflatoxicosis. A subsample of the peanuts contained 77 ppm aflatoxin. More than a few parts per million of aflatoxin are seldom found in nature. Clearly, abortion in these cows was secondary to acute toxicant-induced alteration in maternal homeostasis. No empirical evidence is available to suggest that aflatoxin doses below the acute maternally toxic threshold are capable of a direct abortifacient effect.

POLYBROMINATED BIPHENYLS

The appearance of polybrominated biphenyls (PBBs) in 1973 in cattle feed was a dramatic event receiving widespread media attention. The source of these compounds was from accidental incorporation of a flame retardant into cattle rations.[77] Adverse effects on reproduction included prolonged gestation and the resulting dystocia, poor udder development, septic metritis, and death of newborn calves.

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CHAPTER 55

Dropsical Conditions Affecting Pregnancy

SIMON F. PEEK

Hydrallantois and **hydramnios**, hydrops of the allantois and the amnion, respectively, are the two most common causes of dropsy of the fetal membranes and fetus in cattle. Other, less common causes include edema of the chorioallantois, fetal anasarca, and fetal edema with ascites and hydrothorax.[1] In the largest published retrospective study of hydrops to date, Vandeplassche and associates documented that of a total of 60 cases, hydroallantois accounted for 88%, 5% of the cases were hydramnios, and 7% involved both compartments.[2]

HYDRALLANTOIS

Hydrallantois is the single pathologic factor present in 85% to 90% of dropsical conditions in the bovine.[1,2] Placental dysfunction is thought to be the cause of hydrallantois, although this condition is observed with both uterine and placental disease. Adventitious placentation commonly is present (Fig. 55-1), and the number of caruncles also may be deficient. This deficiency may be due to a congenital lack of development or to uterine disease acquired later in life. A reduction in the number of caruncles also has been noted with hydrallantois, and those that are present tend to be hypertrophied.[3] The condition is seen sporadically in both dairy and beef cows, and dams carrying multiple fetuses are at greater risk for hydrallantois. An increased prevalence of hydrallantois has been noted in recipient cattle carrying fetuses produced by in vitro fertilization and by transgenic and cloning technologies.[4,5]

The structural and functional changes in the chorioallantois that lead to hydrallantois result in the excessive production of a transudative fluid that resembles plasma.[1] In severe hydrallantois, the accumulated fluid volume may reach 150 to 250 L, and the combined weight of the dropsical fluids, membranes, and fetus can exceed 225 kg.[3] Fluid composition studies comparing allantoic fluid from normal cows in late gestation and from those suffering from hydrallantois have demonstrated marked differences in sodium, potassium, chloride, and creatinine levels.[2,6,7] In hydrallantois, the levels of these electrolytes are much higher, more closely resembling plasma levels.[8] In addition, lower peripheral plasma concentrations of estradiol have been observed in cows suffering from hydrallantois.[7] The exact role of estrogen deficiency in the maintenance of fetal membrane permeability and its effect on allantoic and amniotic fluid volume in the cow remains uncertain. Studies in pigs and ovariectomized sheep, however, point to a link between the estrogen-to-progesterone ratio and fetal fluid volume.[8,9]

The clinical signs associated with hydrallantois vary with the volume of fluid at presentation. Abdominal distention rarely has been noted as early as the fifth month of gestation.[3] The typical case of hydrallantois, however, is characterized by a larger than normal accumulation of allantoic fluid during a 5- to 20-day period in the last trimester of pregnancy[3] (Fig. 55-2). Mild cases may remain undiagnosed or may be suspected only at parturition when an abnormally large volume of fetal fluids is expelled. In such instances, the producer may have assumed that the dam was carrying twins. In the more severe cases, progressive abdominal distention during the last 4 to 6 weeks of pregnancy worsens to such an extent as to decrease appetite and cause difficulty moving or rising.[10] Progressive maternal tachycardia, anxiety, reduced appetite, and dehydration should be anticipated with untreated hydrallantois. Recumbency and metabolic problems, particularly ketosis, are therefore potential complications associated with advanced cases of hydrallantois, and these will be even more likely should the condition be associated with multiple fetuses. The combined weight of the fetus(es) and allantoic fluid also may result in either prepubic tendon rupture or secondary ventral abdominal muscle herniation.

A definitive diagnosis of hydrallantois can be obtained by rectal palpation. The external appearance of the cow may suggest the presence of vagal indigestion, but rectal palpation will quickly distinguish between the two conditions. In hydrallantois, the abdominal cavity will be dominated by the fluid-filled uterus, frequently precluding palpation of other structures, whereas vagal indigestion will be characterized by a prominent, enlarged L- or V-shaped rumen. The fetus and placentomes usually are not palpable in cases of hydrallantois, and it is therefore difficult to ascertain the viability of the fetus without fetal heart rate measurement. Evaluation of fetal number and viability is best performed by transabdominal ultrasound examination with a 2.5- or 5-MHz sector scanner.

HYDRAMNIOS

Hydramnios causes approximately 10% of the cases of hydrops of the fetal membranes in cows.[1] The condition is characterized by gradual accumulation of excessive amniotic fluid, with progressive abdominal enlargement in the dam during the last trimester of pregnancy. The abdominal distention typically is slower to develop than

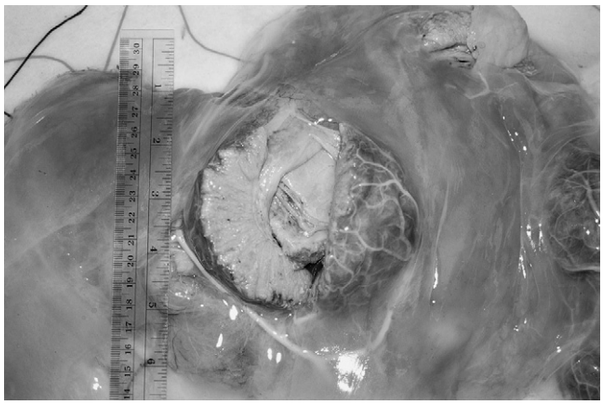

Fig. 55-1 Abnormal cotyledon from the placenta of a cow with hydrallantois (8 months of gestation). The cotyledon, measuring 5 inches in diameter, has been sectioned; note the excessive edema within the intercotyledonary placenta.

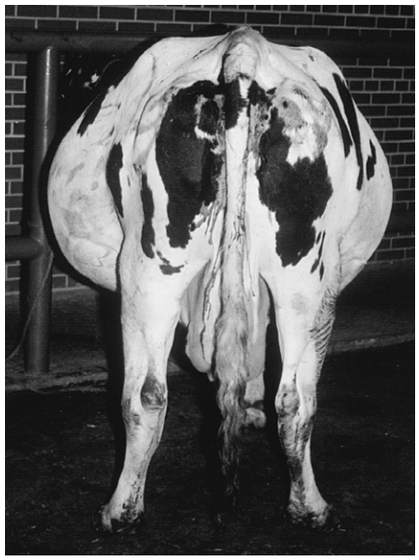

Fig. 55-2 Severe bilateral abdominal distention secondary to hydrallantois is evident when this adult Holstein cow is viewed from the rear. (Courtesy of Dr. W. C. Rebhun.)

in hydrallantois.[3] Hydrops of the amnion usually is the result of an abnormal fetus and is therefore considered a fetal problem, whereas hydrallantois is due to maternal abnormalities of placentation. From midgestation to term, amniotic fluid normally is swallowed or inhaled into the large bronchi of the fetus and subsequently absorbed.[11] Hydramnios results from fetal abnormalities that prevent swallowing or intestinal transport of amniotic fluids.[1] During late gestation, the volume of normal amniotic fluid reaches between 3.8 and 7.6 L; however, in hydramnios, the volume will increase to 19 to 114 L. The fluid is viscous and syrupy in consistency.[1]

Hydramnios can be associated with both genetic and nongenetic fetal abnormalities. Certain fetal anomalies inherited as autosomal recessive traits have been associated with hydramnios in specific breeds of cattle:

- Dexter cattle pregnant with bulldog calves. These fetuses usually are aborted between 5 and 8 months of gestation.
- Angus cattle pregnant with brachygnathic calves whose skeletons also lack marrow cavities. Affected cows demonstrate abdominal enlargement in the last month of pregnancy.
- Red Danish cattle pregnant with muscle contracture monsters
- Guernsey cattle pregnant with calves suffering from pituitary hypoplasia or aplasia. Prolonged gestational length also is seen in this condition owing to the abnormal fetal pituitary-adrenal axis.
- Hereford cattle pregnant with hydrocephalic calves.[1]

The nongenetic causes of hydramnios in cattle include those anomalies that impair the ability of the fetus to swallow amniotic fluid, such as schistosomus reflexus. Hydramnios also has been reported in hybrid crosses between American bison and domestic cattle.[11]

TREATMENT OF HYDRALLANTOIS AND HYDRAMNIOS

The decision whether to treat a cow suffering from hydrallantois or hydramnios should always be tempered by practical considerations regarding the likelihood of success and the etiology of the specific condition. Salvaging the cow for slaughter should be considered in all cases of hydrops in the cow.[1,3,11] The cause of hydrallantois is uterine or placental disease, or both, so the likelihood of recurrence should be considered.[3] In cases of hydramnios, an inherited etiology should be considered and the fact that the fetus most likely will be anomalous should be borne in mind. If the fetal anomaly is due to an established autosomal recessive disorder, both the dam and the sire will be carriers.[1,3]

The severity of the condition at presentation also should play an important role in decision making. In severe cases of hydrallantois in which the patient is already recumbent and unable to rise, or prepubic tendon or abdominal wall rupture has already occurred, the prognosis becomes increasingly grave, and immediate slaughter should be considered. In dairy cattle, because individual animals are in negative energy balance and often within the last trimester at the time of diagnosis, they may never obtain projected production levels despite successful termination of a pathologic pregnancy. For the aforementioned reasons, salvage usually is considered unless the cow or the fetus is particularly valuable and the pregnancy is within 2 to 3 weeks of term.[10]

If the decision is made to treat the condition, several options exist. Most clinicians agree that elective cesarean section is rarely as successful as induced parturition.[3,10,11] Uterine atony due to overdistention, retained fetal membranes, and severe metritis are complications that should always be anticipated after either induced parturition or elective surgery. Debate exists among different authors about whether hydrops patients are markedly dehydrated

or not; some sources support the hypothesis,[1,10] whereas others refute it.[11] The induction of parturition, however, will result in a slower release of uterine fluids than the rapid decompression that occurs after surgery. The rapid release of up to 250 L of allantoic fluid would certainly seem to predispose the patient to hypovolemic shock subsequent to compartmental shifting into the splanchnic pool.[11] Some clinicians advocate the use of a trocar or plastic tube inserted percutaneously through the abdominal and uterine walls to draw off fluid gradually during a 24-hour period before elective cesarean.[6] This procedure will predispose the cow to the development of peritonitis and/or metritis, however, and whether this maneuver actually allows a significant volume of fluid to drain is uncertain.[3] Drainage also can be attempted transcervically if the cervix is relaxed enough to permit passage of a suitable catheter. In cases of hydrallantois treated by cesarean section, some authors have observed that the uterus may continue to fill with a significant volume of transudative fluid for up to 48 hours after surgery.[1]

To induce either abortion or calving, 25 to 35 mg of prostaglandin $F_{2\alpha}$ can be injected intramuscularly either alone or in combination with dexamethasone.[3] An alternative regimen involves the administration of 10 to 20 mg of flumethasone intramuscularly. Some clinicians advocate the use of 6 to 8 mg of estradiol cypionate intramuscularly once daily until parturition to relax the cervix and caudal reproductive tract. The combination of a prostaglandin and an abortifacient steroid reliably induces parturition in late gestation in a normal cow within 24 to 48 hours, but the response can be rather more unpredictable in cases of hydrops.

After induction of parturition, the cow should be carefully monitored and regularly examined for signs of labor. Periodic vaginal examination is important to ascertain the progress of cervical dilation and to rupture the fetal membranes as soon as possible.[3] Once the fetus can be palpated per vagina, it should be assessed for size, position, and the presence of any anomalies that may hinder vaginal delivery. In many cases of hydrallantois, parturition may not progress beyond this point as a result of uterine atony, and assistance will be required to complete delivery.

After induction, the cow should be kept in a deeply bedded stall with good footing, to limit the chances of catastrophic musculoskeletal injury associated with parturition. If dehydration is suspected or if the cow has evidence of other metabolic complications (ketosis, hypocalcemia), then supportive polyionic intravenous fluids augmented with dextrose and calcium are indicated. In a field situation, acute hypovolemic shock can be addressed with smaller volumes of hypertonic saline (2–3 L of 7% sodium chloride given intravenously, followed by access to water and electrolyte solutions). In dairy cows, milking should be initiated as soon as the calf is delivered, although it often can be challenging to achieve projected production levels in cows that have experienced a dropsical pregnancy. A majority of fetuses will be small, nonviable, or anomalous, but in the rare case of an apparently viable calf, colostrum must be provided from an alternative source, because the dam rarely has normal colostrum. With exceptionally valuable calves it is wise to consider plasma administration, because colostrum absorption may be compromised by precocious gut closure induced by chronic in utero swallowing of excessive fetal fluid.

The prognosis for individual animals suffering from hydrallantois, particularly if the condition is advanced at the time of diagnosis, is much poorer for life and future fertility than is the case with hydramnios. Retained fetal membranes and potentially severe metritis should be anticipated with both conditions, but especially with hydrallantois, particularly if parturition is induced or the cervix is still significantly closed at the time of elective cesarean section. Prophylactic antibiotics should be instituted if the cow is not being sold for slaughter.

OTHER DROPSICAL CONDITIONS OF CATTLE

Dropsical conditions other than hydrallantois and hydramnios are very rare but include the following:

Fetal ascites. This condition has been associated with both infectious and noninfectious fetal pathology. Fetal ascites and fetal and placental edema can be seen with intrauterine fetal death and autolysis from whatever cause, and the degree of fetal enlargement dictates whether or not dystocia results.[1]

Edema of the chorioallantois. Excessive edema of the chorioallantois has been classically associated with *Brucella abortus* infection.[1]

Fetal anasarca or excessive fetal edema. Both of these conditions can be seen in isolation or in combination with hydrallantois or hydramnios.[1] Fetal anasarca can be an inherited condition in the Ayrshire breed as an autosomal recessive disorder.[12]

In all of these conditions, with the possible exception of edema of the chorioallantois, abdominal enlargement usually is not noted.[1]

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CHAPTER 56

Effects of Environment on Bovine Reproduction

PETER JAMES HANSEN

> Whoever would study medicine aright must first learn of the following subjects. First, he must consider the effect of each of the seasons of the year and the differences between them.
>
> HIPPOCRATES (c. 400 BC), *Airs, Waters, Places*

Originally domesticated in the Near East around 8000 years ago, domestic cattle have become distributed throughout most of the world. The rate of geographic dispersion of cattle has been slow until recently, and distinct genetic populations of cattle have arisen that are adapted to specific environments. Beginning in the latter half of the 19th century, and progressing rapidly in the 20th century to the present, however, technologic advances in transportation and management practices have led to rapid changes in the environment in which cattle are reared and in the genetic composition of cattle. These advances involved geographic change in location of specific breeds, management practices that confer environmental stress, and alterations in the genetics and physiology of cattle that have reduced adaptation to the local environment. As a result, cattle often are placed in environments in which physiologic and productive functions are not optimal. Cattle, like most mammals, are very adaptable animals and possess many homeokinetic mechanisms to maintain critical body functions at the expense of changes in other physiologic functions. Unfortunately, reproduction is one of the physiologic functions that often is most expendable in homeokinetic control systems. In fact, reproductive function is dictated in large part by the environment, and heritability estimates for reproductive traits are low.

At least four general approaches can be taken to manipulate environmental effects on reproduction. First, the environment can be altered to provide cows with conditions more conducive to reproduction. Second, it also is possible to change the cow genetically so that it is more adapted to local environmental conditions. Third, it often is possible to schedule reproductive activity so it occurs when climatic conditions are optimal. Finally, it sometimes is possible to alter the physiology of the cow to modify the effects of environment on reproduction. Successful development of any of these schemes will depend on knowledge of the magnitude of environmental effects and of the physiologic alterations mediating environmental effects. This chapter discusses in detail what is known about these subjects for two environmental inputs—heat stress and photoperiod—and suggests management approaches to enhance reproductive function of cattle through manipulation of these environmental influences. Also provided is some information about other environmental regulators of reproduction.

EFFECTS OF HEAT STRESS ON REPRODUCTIVE FUNCTION

Among the highest physiologic priorities of all homeotherms is maintenance of body temperature. The cow maintains a constant core body temperature of approximately 38.5°C by matching internal heat production with net loss of heat to the environment. In situations leading to hyperthermia (internal heat

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> HIPPOCRATES (c. 400 BC), *Airs, Waters, Places*

Originally domesticated in the Near East around 8000 years ago, domestic cattle have become distributed throughout most of the world. The rate of geographic dispersion of cattle has been slow until recently, and distinct genetic populations of cattle have arisen that are adapted to specific environments. Beginning in the latter half of the 19th century, and progressing rapidly in the 20th century to the present, however, technologic advances in transportation and management practices have led to rapid changes in the environment in which cattle are reared and in the genetic composition of cattle. These advances involved geographic change in location of specific breeds, management practices that confer environmental stress, and alterations in the genetics and physiology of cattle that have reduced adaptation to the local environment. As a result, cattle often are placed in environments in which physiologic and productive functions are not optimal. Cattle, like most mammals, are very adaptable animals and possess many homeokinetic mechanisms to maintain critical body functions at the expense of changes in other physiologic functions. Unfortunately, reproduction is one of the physiologic functions that often is most expendable in homeokinetic control systems. In fact, reproductive function is dictated in large part by the environment, and heritability estimates for reproductive traits are low.

At least four general approaches can be taken to manipulate environmental effects on reproduction. First, the environment can be altered to provide cows with conditions more conducive to reproduction. Second, it also is possible to change the cow genetically so that it is more adapted to local environmental conditions. Third, it often is possible to schedule reproductive activity so it occurs when climatic conditions are optimal. Finally, it sometimes is possible to alter the physiology of the cow to modify the effects of environment on reproduction. Successful development of any of these schemes will depend on knowledge of the magnitude of environmental effects and of the physiologic alterations mediating environmental effects. This chapter discusses in detail what is known about these subjects for two environmental inputs—heat stress and photoperiod—and suggests management approaches to enhance reproductive function of cattle through manipulation of these environmental influences. Also provided is some information about other environmental regulators of reproduction.

EFFECTS OF HEAT STRESS ON REPRODUCTIVE FUNCTION

Among the highest physiologic priorities of all homeotherms is maintenance of body temperature. The cow maintains a constant core body temperature of approximately 38.5°C by matching internal heat production with net loss of heat to the environment. In situations leading to hyperthermia (internal heat

production greater than net loss of heat), thermoregulatory regions of the central nervous system engage physiologic systems to reduce internal heat production and increase net heat flow from the body. Several physiologic changes that stabilize body temperature are deleterious to reproduction. In addition, the hyperthermia experienced by heat-stressed cows can itself compromise reproductive function. Taken together, these effects lead to a severe reduction in reproductive performance of cattle.

All cattle are at risk of becoming hyperthermic, but the problem of heat stress is greatest in lactating dairy cattle populations. Genetic selection for milk yield has produced an animal with high internal heat production, thus increasing susceptibility to hyperthermia. In lactating cows, the upper critical temperature (the ambient temperature at which hyperthermia occurs) is as low as 24° to 27° C. Effects of heat stress are therefore not limited to the tropics but sometimes occur during the summer in temperate zones. By contrast, many beef breeds are adapted for tropical or semitropical conditions. Indeed, some reports indicate that reproductive function of Zebu cattle is highest in summer and lowest in winter.

Detection of Estrus

As shown in Figure 56-1, ability to detect estrus in *Bos taurus* declines during periods of heat stress. This decline occurs in large part because of a reduction in duration of estrous behavior. In contrast with *B. taurus*, evidence for decreased duration of estrus in *Bos indicus* by heat stress is scarce. Under Florida conditions, estrus in Brahman heifers averaged 8.6 ± 2.3 hours in spring, 5.5 ± 1.2 hours

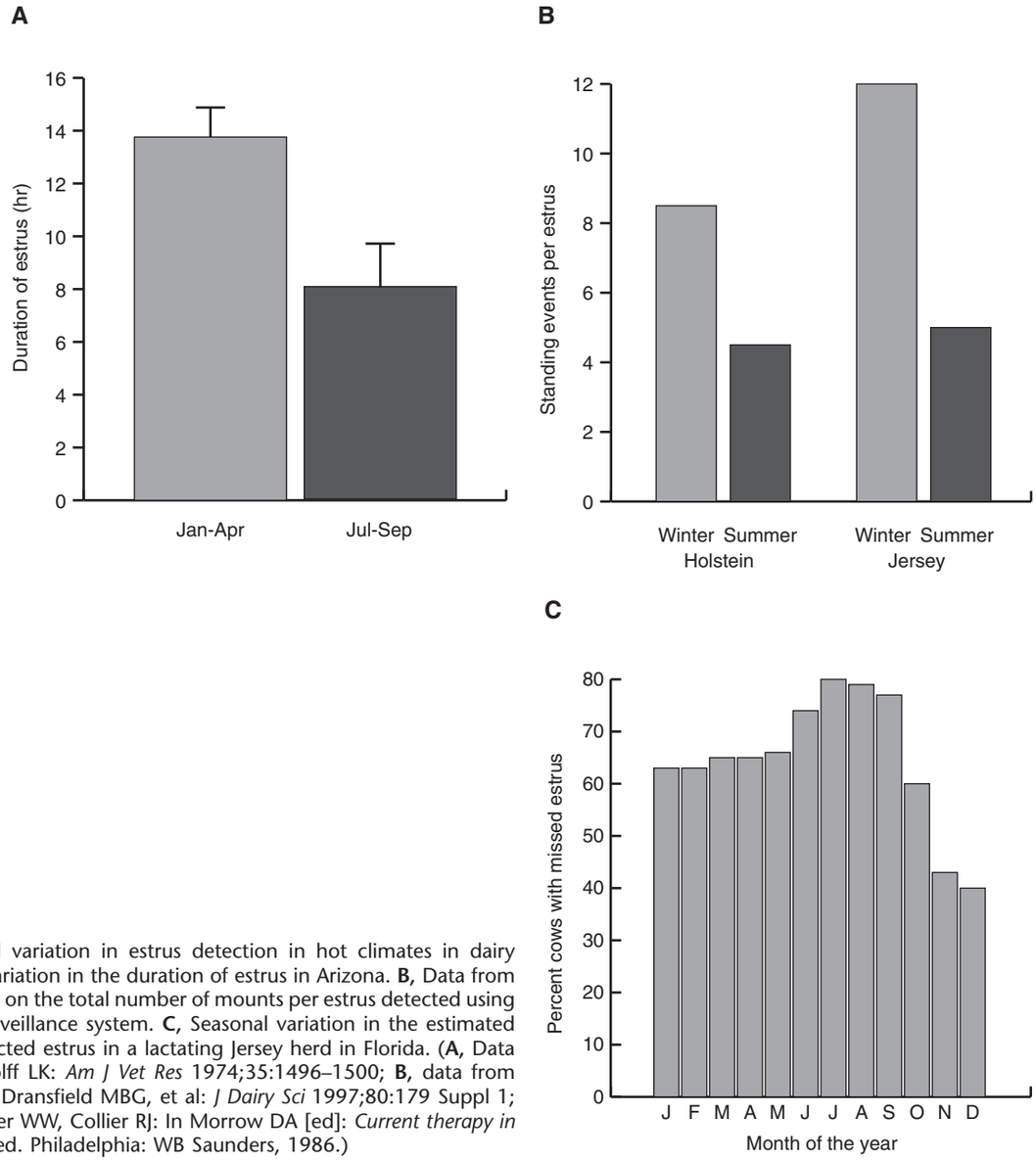

Fig. 56-1 Seasonal variation in estrus detection in hot climates in dairy cattle. **A,** Seasonal variation in the duration of estrus in Arizona. **B,** Data from dairy cows in Virginia on the total number of mounts per estrus detected using a radiotelemetric surveillance system. **C,** Seasonal variation in the estimated frequency of undetected estrus in a lactating Jersey herd in Florida. (**A,** Data from Monty DE, Wolff LK: *Am J Vet Res* 1974;35:1496–1500; **B,** data from Nebel RL, Jobst SM, Dransfield MBG, et al: *J Dairy Sci* 1997;80:179 Suppl 1; **C,** data from Thatcher WW, Collier RJ: In Morrow DA [ed]: *Current therapy in theriogenology*, 2nd ed. Philadelphia: WB Saunders, 1986.)

in summer and 5.4 ± 0.5 hours in fall and 6.8 ± 1.6 hours in winter.[1] In this same study, under conditions in which variation in feed availability was minimal, the proportion of ovulations without detected estrus was greater in winter (59.7%) than in summer (40.3%).

Reduced estrous behavior probably is related, at least in part, to reduced locomotor activity experienced by heat-stressed animals. An endocrine cause also is possible, because heat stress has been reported to reduce peripheral concentrations of estradiol-17β around the time of estrus in some studies. Heat stress also has been observed to decrease gonadotropin responses to injection of gonadotropin-releasing hormone (GnRH) in cows with low circulating concentrations of estradiol-17β.

Effects of Heat Stress on Establishment of Pregnancy

Data in Figure 56-2 illustrate the marked seasonal depression in pregnancy rates per insemination of artificially inseminated dairy cattle in warm months of the year. This depression is most apparent in regions of the world with hot climates but can occur in more temperate areas also. Heat stress is the major environmental factor responsible for lowered pregnancy rates in the summer. Experimental exposure of cattle to heat stress reduced pregnancy rate and embryonic survival, whereas provision of cooling during the summer improved the frequency of cows' becoming pregnant after insemination (Table 56-1). Moreover, the magnitude of the depression in pregnancy rate is proportional to the degree of hyperthermia; this has been demonstrated for both dairy and beef cows.

It is important to define the phases of the reproductive process leading to fertilization and embryonic development that are sensitive to maternal hyperthermia, because together these phases dictate the critical windows in which cows must be kept homeothermic to maintain high pregnancy rates. Disruption of the reproductive process can occur quite early—during oocyte growth or maturation. Although little seasonal effect on fertilization rate for oocytes subjected to in vitro fertilization (IVF) has been observed, fertilized oocytes collected from cows during the summer are less likely to give rise to an embryo capable of development to the blastocyst stage than are embryos collected during the winter. Seasonal variation in performance of embryo production systems based on

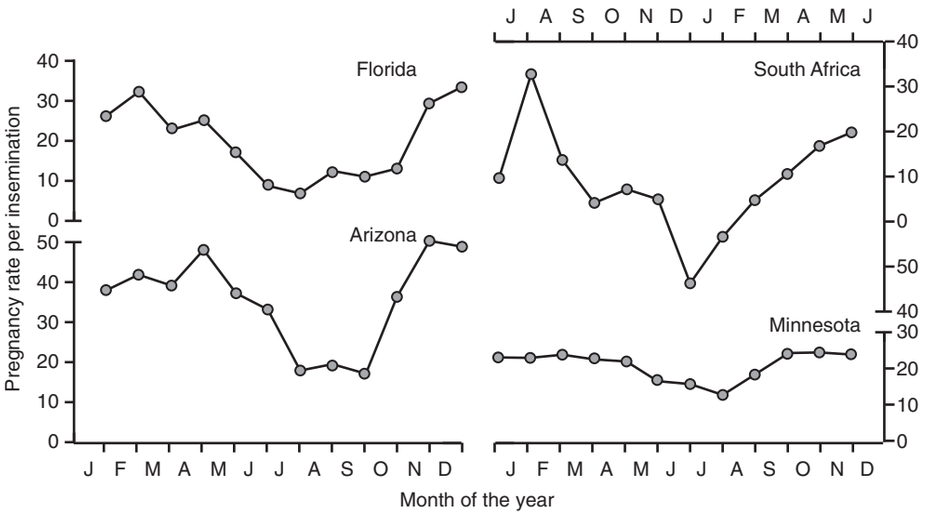

Fig. 56-2 Seasonal variation in pregnancy rates per insemination (i.e., conception rates) for lactating dairy cows in Arizona, Florida, South Africa, and Minnesota. (Data for Arizona: from Monty DE, Wolff LK: *Am J Vet Res* 1974;35:1496–1500; for Florida: from Cavestany D, El-Wishy AB, Foote RH: *J Dairy Sci* 1985;68:1471–1478; for South Africa: from Du Preez JH, Terblanche SJ, Giesecke WH, et al: *Theriogenology* 1991;35: 1039–1049; and for Minnesota: from Udompraset P, Williamson NB: *Theriogenology* 1987;28: 323–336.)

Table **56-1**

Cooling during Heat Stress and Pregnancy Rates in Lactating Dairy Cows

			NO. PREGNANT/NO. BREEDINGS (%)		
Study*	**Location**	**Type of Cooling**	**Control**	**Cooled**	**P Value**
1	Arizona	Evaporative cooling vs. shade (control)	24/69 (35%)	36/62 (58%)	—
2	Florida	Refrigeration vs. no shade (control)	48/170 (28%)	53/135 (39%)	NS
3	Florida	Shade vs. no shade (control)	19/75 (25%)	24/54 (44%)	0.05
4	Israel	Fans, sprinklers and shade vs. shade (control)	14/70 (20%)	32/56 (57%)	0.01

*Data for Study 1 from Roman-Ponce H, Thatcher WW, Buffington DE, et al: *J Dairy Sci* 1977;60:424–430; for Study 2 from Stott GH, Wiersma F, Woods JM: *J Am Vet Med Assoc* 1972;161:1369–1375; for Study 3 from Thatcher WW, Gwazdauskas FC, Wilcox CJ, et al: *J Dairy Sci* 1974;57:304–307; and for Study 4 from Wolfenson D, Flamenbaum I, Berman A: *J Dairy Sci* 1988;71:3497–3504.
NS, not significant.

IVF has been observed even in a relatively cool region like Wisconsin. Additional evidence from Israel suggests that oocyte quality gradually improves as season progresses from early to late autumn, as supported by data from studies in which cows were subjected to multiple oocyte recoveries.

The periovulatory period represents another period during which the establishment of pregnancy can be disrupted by heat stress. For example, heat stress of superovulated cows for 10 hours beginning at the onset of estrus had no effect on fertilization rate but reduced the proportion of normal embryos recovered on day 7 after estrus. This effect of heat stress was not caused by effects on spermatozoa within the reproductive tract, because insemination was not performed until body temperatures of heat-stressed cows had returned to normal. Rather, heat stress altered the oocyte or the reproductive tract so that normal embryonic development was compromised.

In another study, it was demonstrated that heat stress of superovulated cows reduced development and viability of embryos if cows were exposed to heat stress at day 1 after estrus but not if heat stress was given on day 3, 5, or 7 after estrus (Fig. 56-3). Moreover, the reduction in development to the blastocyst stage caused by exposure of cultured embryos to elevated temperature declined as embryos proceeded through development (see Fig. 56-3). Thus, embryos become more resistant to effects of heat stress as pregnancy proceeds. Nonetheless, the embryo can still be damaged by severe heat stress even late in development. For example, heat stress of beef cows from days 8 to 16 of pregnancy reduced conceptus size at day 17.

Causes of Heat Stress–Associated Pregnancy Failure

Potentially, heat stress could cause pregnancy loss by exerting actions on either the oocyte, embryo, or reproductive tract. Furthermore, disruption of pregnancy could occur because of direct effects of elevated temperature on cellular function or as an indirect consequence of physiologic changes for regulation of body temperature.

Heat stress could compromise oocyte competence by at least three possible mechanisms: (1) Disruption in patterns of folliculogenesis could lead to ovulation of an aged oocyte with lowered potential for fertilization. Consistent with this idea is the finding that heat stress beginning at day 1 of the estrous cycle caused earlier emergence of the dominant follicle of the second follicular wave. Such an effect could conceivably alter the quality of the oocyte ovulated at the subsequent estrus, because oocytes from persistent dominant follicles have reduced competence. This mechanism must not be the only one operational, however, because it does not explain why season (and presumably heat stress) adversely affects multiple oocytes in the cohort of follicles ovulated after superovulation, collected by transvaginal ultrasound–guided recovery, or aspirated from excised ovaries. (2) Heat stress also has been reported to affect follicular steroidogenesis.

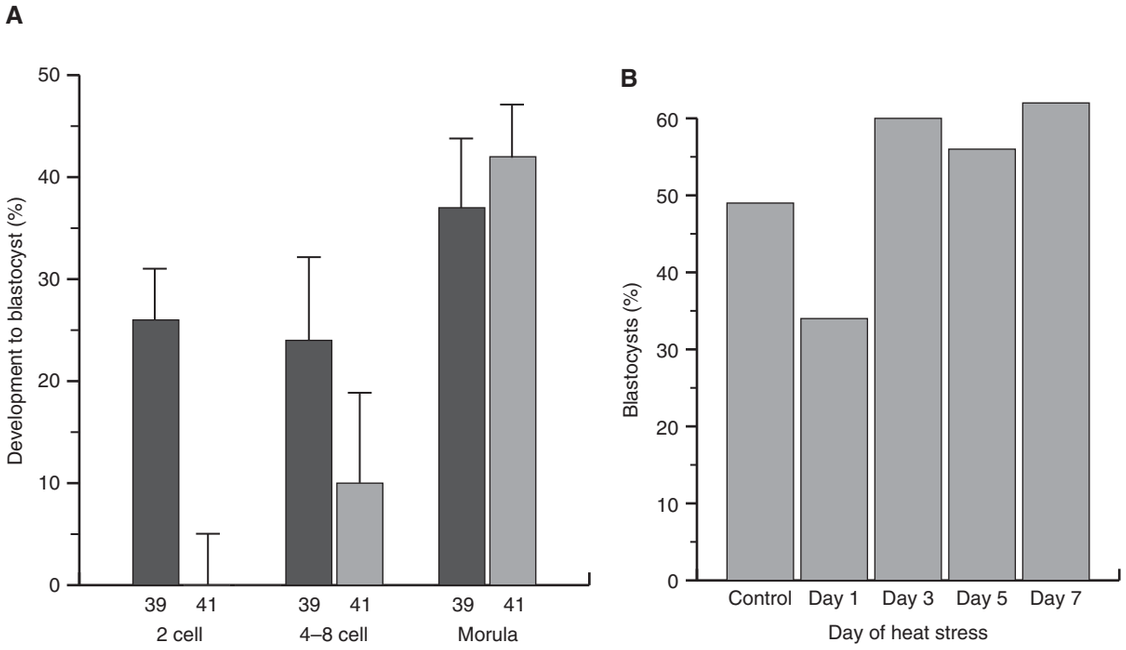

Fig. 56-3 Developmental changes in resistance of bovine embryos to heat shock. **A,** *Left panel*: Effect of heat stress on day 1, 3, 5, and 7 of pregnancy (day 0 = estrus) in superovulated cows on the proportion of embryos recovered as blastocysts. **B,** *Left panel*: Data illustrating how stage of embryonic development affects response of embryos to an elevated temperature of 41°C for 12 hours, compared with control conditions of 39°C. (**A,** *Left panel*: Data from Ealy AD, Drost M, Hansen PJ: *J Dairy Sci* 1993;76:2899–2905; **B,** *left panel*: data from Edwards JL, Hansen PJ: *Mol Reprod Dev* 1997;46:138–145, 1997.)

Perhaps alterations in the steroidal environment disrupt oocyte development. (3) Finally, oocytes may be similar to male germ cells in that they have heightened sensitivity to elevated temperature. Unlike most cells, the oocyte in developing follicles is transcriptionally inactive after it reaches a diameter of approximately 110μm (i.e., at about the 3-mm follicle stage). This means that the range of cellular adjustments to elevated temperature that are possible in the oocyte are limited to those not involving transcription. For example, bovine oocytes cannot undergo increased synthesis of heat shock protein 70 (HSP70) in response to elevated temperature.

Sensitivity to elevated temperature persists for several cleavage divisions after fertilization, with the embryo acquiring increased resistance to heat shock as it proceeds through development. Thus, the increased resistance of cows to heat stress as pregnancy proceeds may reflect developmental changes in the embryo that lead to greater cellular resistance to elevated temperatures (see Fig. 56-3). The molecular basis for increased embryonic thermotolerance is not known. Heat shock protein synthesis in embryos exposed to elevated temperature occurs as early as the four-cell stage, and other biochemical changes may therefore also be important.

Hyperthermia also may disrupt endometrial function directly, as has been reported for cultured endometrial and oviductal explants. Although protein secretion by endometrium is more resistant to the disruptive effects of heat shock than conceptus tissue, endometrial synthesis of two heat shock proteins, HSP90 and HSP70, is induced by heat shock. These proteins are components of the progesterone receptor complex, and increased synthesis of HSP70 and HSP90 in endometrium could conceivably inhibit endometrial responsiveness to progesterone. Evidence that progesterone support of the uterus is reduced by heat stress as a result of decreased circulating concentrations of progesterone is highly equivocal and may depend on the duration and severity of stress, as well as the nature of concomitant changes in blood volume. Heat stress has been reported to reduce uterine blood flow, and such an effect could reduce delivery of nutrients and hormones to the uterus.

The process by which the embryo causes maintenance of the corpus luteum also is potentially sensitive to disruption by heat stress. Culture at 43° C reduced secretion of the antiluteolytic hormone interferon-τ by day 17 conceptuses. Heat shock also increased release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 from endometrial explants and uterine production of $PGF_{2\alpha}$ in response to oxytocin.

Effects of Heat Stress in Late Pregnancy and the Postpartum Period

Abortion caused by heat stress is rare, but placental function is compromised by heat stress in the last third of pregnancy. Thus, in one study, secretion of the placental hormone estrone sulfate, placental size, and calf birth weight were reduced in the summer or by experimental heat stress (Table 56-2). These effects of heat stress probably are caused primarily by the reduction in blood flow to the placenta that occurs with heat stress. In one study in California, calf mortality in the summer increased as environmental temperature increased. This relationship could reflect the lower calf birth weights caused by heat stress, as well as reduced immunoglobulin transfer to the neonate.

Mammogenesis is dependent on placental function, so it would be expected that heat stress during late pregnancy compromises subsequent milk yield. Indeed,

Table **56-2**

Heat Stress during Late Gestation and Calf Birth Weight and Subsequent Milk Yield in Dairy Cows

		MILK YIELD		CALF BIRTH WEIGHT (KG)		
Study	**Location**	**Control**	**Cooled**	**Control**	**Cooled**	***P* Value**
1[a]	Florida	***100-day milk yield (kg)***		36.6 ± 0.7	39.7 ± 0.7	0.05
		2556 ± 302	2672 ± 302			NS
		305-day milk yield (kg)[b]				
		5948 ± 505	6758 ± 505			NS
2[c]	Israel	***150-day milk yield (kg/day)***		40.6 ± 0.9	43.2 ± 0.8	0.01
		37.2 ± 0.9	40.7 ± 1.0			0.01
		150-day FCM (kg/day)				
		32.6 ± 0.7	35.6 ± 0.8			0.01

[a]Beginning in June, Holsteins cows and heifers were either managed in a no-shade environment (control) or given shade during the last trimester of pregnancy. Data from Collier RJ, Doelger SG, Head HH, et al: *J Anim Sci* 1982;54:309–319.
[b]Milk yield adjusted for age, month of calving, and estimated relative producing ability.
[c]From June to October, Israeli-Holstein cows were either given access to shade (control) or given shade or cooled with fans and sprinklers during the dry period. Data from Wolfenson D, Flamenbaum I, Berman A: *J Dairy Sci* 1988;71:809–818.
FCM, fat-corrected milk yield; NS, not significant.

cooling heat-stressed cows during the dry period can sometimes increase subsequent milk yield (see Table 56-2).

Consequences of heat stress for reproductive function in the cow during the postpartum period have not been extensively studied. The incidence of retained placenta was higher in the summer in a study in Georgia. Heat stress during late pregnancy increased postpartum uterine output of $PGF_{2\alpha}$ but had no effect on resumption of estrous cycles. Similar studies have not examined this effect in beef cattle, although suckling could change the relationship between heat stress and postpartum endocrine function.

Effects of Heat Stress on the Bull

Many mammals have evolved so that male gametogenesis is unable to occur at temperatures characteristic of the body core—heat stress for as little as 12 hours disrupts spermatogenesis in the bull. The stage of spermatogenesis that is most susceptible to elevated temperature is the primary spermatocyte, although damage to B spermatogonia can occur in bulls and prolonged exposure to heat damage can damage dividing spermatocytes and spermatids. Mammals have evolved an intricate anatomic and physiologic system for local thermoregulation of the testis that involves placement of the testis outside the body cavity, exchange of heat between the body core and testis through countercurrent mechanisms in the pampiniform plexus, and regulation of heat loss to the surrounding air by means of muscular control of the placement of the testis relative to the body and the surface area of the scrotum and by sweating. As a result, testicular temperature is approximately 5°C below core body temperature. Spermatogenesis is disrupted when either the thermoregulatory system that maintains testicular temperature is disrupted or an elevation in body temperature caused by fever or heat stress raises the temperature of the blood reaching the testis. Effects of disruption in sperm production include decreased sperm numbers, decreased sperm motility, and increased numbers of abnormal sperm. Some reports have suggested that spermatozoa from heat-stressed bulls are less able to withstand freezing and to bind heparin.

The timing of changes in sperm production after the onset and termination of heat stress is illustrated in Figure 56-4. There is a delay of about 2 weeks between heat stress and the onset of the first alterations in sperm output, because the primary spermatocyte is the most susceptible germ cell. The process of spermatogenesis takes approximately 54 days in bulls, and effects of heat stress on sperm output persist for 7 to 8 weeks after the end of heat stress.

Alterations in endocrine function caused by heat stress are much less severe than effects on spermatogenesis. Transferring bulls from 21° to 35.5°C caused a decrease in testosterone concentrations in one study, whereas heat stress of 34°C caused a transitory decrease in luteinizing hormone (LH) secretion without any effect on testosterone concentrations in another study.

Although artificial insemination bypasses many of the effects of heat stress on the bull, the possibility remains that spermatozoa become heat shocked when they are placed in the reproductive tract of a hyperthermic female. Exposure of frozen thawed spermatozoa to 41° to 42°C had little effect on motility and no effect on fertilizing ability. It is possible, however, that embryos formed by fertilization of heat-shocked sperm are compromised in ability to develop, as was concluded from studies in the rabbit.

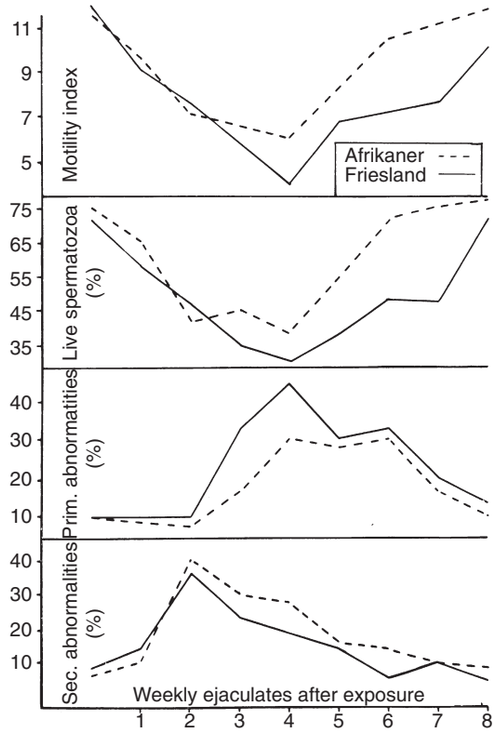

Fig. 56-4 Time-dependent changes in characteristics of ejaculates collected from bulls exposed to a 6-day period of heat stress (40°C for 12 hours per day). Ejaculates were collected after completion of heat stress and at weekly intervals thereafter. (From Skinner JD, Louw GN: *J Appl Physiol* 1966;21: 1784–1790, with permission of *Journal of Applied Physiology*.)

MEASURING THE MAGNITUDE OF HEAT STRESS

Successful animal thermoregulation involves the balance of heat production and heat loss. Environmental variables determining the degree to which thermoregulation is possible include air temperature, humidity, wind speed, and solar radiation. The degree of heat stress depends on all of these variables. The best way to determine the magnitude of heat stress in a simple and accurate manner is through use of the black globe thermometer. This is a device in which a thermometer is placed inside a round black vessel—it can be as simple as a toilet bowl float painted black. The thermometer does not undergo evaporative cooling (unless wetted), but otherwise the temperature inside the float depends on the same environmental factors that determine the degree of heat stress that cows experience—air temperature, solar radiation, and wind speed. Effect of humidity on thermal balance

of the cow can be considered through use of temperature-humidity indexes or black globe temperature-humidity indexes. One black globe temperature-humidity index (BGTHI) used by Buffington and co-workers[2] is as follows:

$$BGTHI = BGT + 0.36T_{dp} + 41.5$$

where T_{dp} is dewpoint temperature and all temperatures are in degrees centigrade.

Meteorologic measurements are useful in assessing heat stress but do not always reflect the change in body temperature experienced by a cow in response to heat stress. This is because the body temperature change depends on resistance to heat flow and internal heat production. Effects of heat stress on cattle are modified by breed, coat color, lactation, drugs such as xylazine and bovine somatotropin, and exposure to flies. Accordingly, direct measurements of body temperature probably are the best indicator of the degree to which physiologic functions of the cow are depressed by heat stress. In lactating dairy cows, a rise in uterine temperature by 0.5°C above normal on the day of insemination was associated with a 12.8% decline in pregnancy rate.[3] The genetic correlation between rectal temperature and calving rate in beef cattle in Queensland was 0.76.[4]

SCHEMES TO PREVENT EFFECTS OF HEAT STRESS ON REPRODUCTION

Estrus Detection

Cooling cows around the time of anticipated estrus can increase estrus detection. Using such an approach, Her and colleagues[5] observed that the proportion of cows detected in estrus was higher for cooled cows (88%) than for noncooled cows (66%), and Ealy and associates[6] reported that 47% of cows cooled with fans and sprinklers were detected in estrus, versus 37% for cows receiving shade alone.

It also is expected that use of estrus detection aids will improve detection of estrus during hot weather. Such systems range from the simple to the complex. Simple systems include application of paint to the tailhead, whereas more complex systems include the HeatWatch system, in which a radiotelemetric pressure transducer is affixed to the cow to transmit information regarding number of times an animal is mounted, and pedometers that measure increased locomotor activity associated with estrus. In a study conducted during the summer in Florida, the proportion of cows detected in estrus within 96 hours after injection of $PGF_{2\alpha}$ was increased by the use of tail chalk from 24% to 43%.[6]

One method for completely eliminating problems of estrus detection is to use an ovulation synchronization protocol that allows timed artificial insemination without the need for estrus detection. The most common system, called the OvSynch method, involves injection of 100 μg GnRH at day 0, 35 mg $PGF_{2\alpha}$ on day 7, and 100 μg GnRH on day 9 and insemination on day 10. Effectiveness of timed artificial insemination during the summer is illustrated in Table 56-3. Timed artificial insemination protocols do not reduce the effect of heat stress on fertility—rates of pregnancy per insemination generally are similar in cows inseminated at a fixed time and in those inseminated at estrus. Because all cows eligible for breeding are inseminated, however, the herd pregnancy rate (the proportion of cows eligible for breeding that become pregnant, calculated as estrus detection rate ×

Table **56-3**

Timed Insemination Protocols for Increasing Pregnancy Rates during Periods of Heat Stress in Lactating Cows[a]

				PREGNANCY RATES		
Study[b]	**Treatment**[c]	***n***	**Interval from Calving to First Service (days)**	**At First Service**	**At Day 90 Post Partum**	**At Day 120 Post Partum**
1	BOE	184	82.4 ± 1.0	12.5 ± 2.5	9.8 ± 2.5	30.4 ± 3.5
	TAI	169	72.4 ± 1.0[d]	13.6 ± 2.6	16.6 ± 2.6[e]	32.7 ± 3.6
2	BOE	35	58.1 ± 1.7	8.6 ± 5.1	14.3 ± 7.2	37.1 ± 8.3
	TAI	35	51.7 ± 1.7[e]	11.4 ± 5.1	34.3 ± 7.1[f]	62.9 ± 8.3[e]
3	$PGF_{2\alpha}$	156	91.0 ± 1.9	4.8 ± 2.5		16.5 ± 3.5
	TAI	148	58.7 ± 2.1[e]	13.9 ± 2.6[e]		27.0 ± 3.6[e]

[a]Holstein herds in Florida. Data represent least-squares means ± SEM.
[b]Studies 1 and 2: Data from Aréchiga CF, Staples CR, McDowell LR, et al: *J Dairy Sci* 1998;81:390–402; study 3: data from de la Sota RL, Burke JM, Risco CA, et al: *Theriogenology* 1998;49:761–770.
[c]BOE, breeding at each observed estrus beginning at day 70 (study 1) or day 50 (study 2) post partum; TAI, timed artificial insemination programmed for day 70 (study 1), day 50 (study 2), or day 60 (study 3), followed by breeding at all observed estrous periods thereafter; $PGF_{2\alpha}$, injection of prostaglandin $F_{2\alpha}$ at 57 days post partum and breeding at all detected estrous periods thereafter.
[d]$P < 0.001$.
[e]$P < 0.05$.
[f]$P < 0.10$ ($P = 0.055$).

pregnancy rate per insemination) increases at fixed times postpartum.

Cooling of Cows to Improve Fertility

Data in Table 56-1 illustrate that altering the environment to reduce the magnitude of heat stress improves fertility in lactating cows. A good review on the design of dairy cattle housing in hot climates is available.[7] The most effective housing systems for cooling lactating cows are those that are designed to maximize the time that cows spend in a cooled environment, achieved through the combined use of shade, fans, and an evaporative cooling system of sprinklers or misters (Fig. 56-5). Although continuous cooling of cattle during hot months provides greatest relief from effects of heat stress, some improvement in pregnancy rates has been achieved by cooling cows during periods when establishment of pregnancy is most susceptible to disruption by heat stress. This approach to improving fertility in the summer has been termed **strategic cooling** and depends on estrous synchronization to control when cows are placed in the cooled environment. In practice, strategic cooling systems have been effective in some experiments but not in others. Averaged across multiple studies, application of strategic cooling from a variable period of 4 to 16 days around the time of estrus increased pregnancy rate from 19% in control animals to 28% in cooled cows.

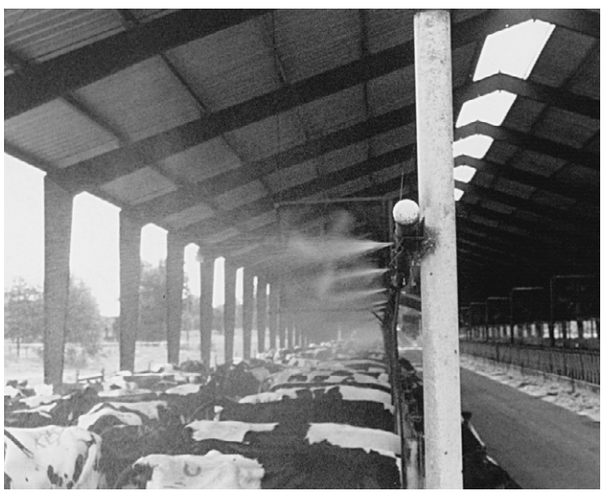

Fig. 56-5 A freestall barn modified to provide evaporative cooling. Facilities such as the one shown here are becoming increasingly common in hot regions. The barn itself provides shade, and additional cooling is provided by sprinklers and fans. The sprinklers are designed to apply water at a rate and drop size sufficient to allow water penetration to reach the skin. Typically, sprinklers are programmed to turn on for 3 minutes on a 15-minute cycle. The fans, which operate continuously, promote evaporation of water from cows and also provide convective cooling. (Courtesy of David Bray, University of Florida.)

Use of Artificial Insemination and Embryo Transfer

Artificial insemination can eliminate infertility caused by heat stress in bulls because semen collected in cool environments can be stored frozen until later use in hot weather. Similarly, embryo transfer can be used to reduce effects of heat stress on the cow. This is so because only embryos that develop to the blastocyst stage and have acceptable morphologic characteristics are transferred. Moreover, embryos subjected to maternal hyperthermia at day 7 (and presumably later) are more resistant to maternal hyperthermia than are embryos at very early stages of development. Several studies conducted in Florida have demonstrated the efficacy of embryo transfer for improving fertility during heat stress (Table 56-4).

One potential limitation to the routine use of embryo transfer in summer is the fact that heat stress can reduce the rate of production of embryos from superovulation and IVF. This limitation can be bypassed by the use of frozen embryos collected at cool periods of the year or in geographic regions where cattle are not susceptible to heat stress. Unfortunately, IVF-derived embryos exhibit poor survival after cryopreservation. Thus, although transfer of fresh embryos produced by superovulation or IVF increased pregnancy rate in summer as compared with artificial insemination, such was not the case when frozen or vitrified IVF-derived embryos were used (see Table 56-4). Production of IVF-derived embryos using oocytes recovered from slaughterhouse ovaries is an inexpensive way of producing large numbers of embryos for transfer into heat-stressed recipients. Widespread use of such embryos, however, probably will depend on improvements in culture systems or cryopreservation protocols that allow high survival rates after freezing.

Genetic Improvement

Distinct breed differences are recognized in effects of heat stress on reproductive function, with reproductive function in *B. indicus* and other thermotolerant breeds being less affected by heat stress than in breeds that evolved in Northern climates. Differences between *B. taurus* and *B. indicus* in thermotolerance are due largely to differences in thermoregulation. In one study, the change in calving rate for each unit increase in rectal temperature was the same for *B. taurus* and *B. indicus* × *B. taurus* crossbreds. Some evidence, however, indicates that breed differences in thermotolerance also exist at the cellular level. Most strikingly, Brahman embryos were found to be more resistant to culture at elevated temperatures than Holstein or Angus embryos. This finding raises the possibility that it may be possible to identify genes that confer cellular thermotolerance and transfer these to breeds of cattle susceptible to heat stress so as to reduce infertility during heat stress.

The relative advantages and disadvantages of crossbreeding between thermotolerant and nontolerant breeds depends on a host of conditions and is beyond the scope of this chapter. Genetic selection to increase thermotolerance also can be practiced but may have undesirable consequences. One trait that contributes to thermotolerance

Table **56-4**

Heat Stress and Pregnancy Rates in Lactating Cows with Different Embryo Production/ Insemination Techniques[a]

Study[b]	Treatment[c]	*n*	Pregnancy Rate (%)
1	AI	524	13.5[d]
	ET, SO, unfrozen embryo	113	29.2
2	AI	84	21.4[e]
	ET, SO, frozen embryo	48	35.4
	ET, IVF, frozen embryo	48	18.8
3	TAI	129	4.3[f]
	TET, IVF, unfrozen embryo	133	17.0
	TET, IVF, frozen embryo	142	7.1
4	TAI	68	6.2[g]
	TET, IVF, unfrozen embryo	33	19.0
	TET, IVF, vitrified embryo	54	6.5

[a]Studies conducted during the summer in herds maintained in Florida.
[b]Study 1: data from Putney DJ, Drost M, Thatcher WW: *Theriogenology* 1989;31:765–778. Study 2: data from Drost M, Ambrose JD, Thatcher MJ, et al: *Theriogenology* 1999;52:1161–1167. Study 3: data from Ambrose JD, Drost M, Monson RL, et al: *J Dairy Sci* 1999;82:2369–2376. Study 4: data from Al-Katanani YM, Drost M, Monson RL, et al: *J Dairy Sci* 2001;84: Suppl 1.
[c]Various embryo production techniques and breeding/insemination protocols were used: AI, artificial insemination after detection of estrus; ET, embryo transfer at 7 days after estrus; IVF, in vitro fertilization; SO, superovulation. TAI (timed AI) protocol: gonadotropin-releasing hormone (GnRH) on day 0, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on day 7, GnRH on day 9, AI at 16 hours after second GnRH. TET (timed ET) protocol: GnRH on day 0, $PGF_{2\alpha}$ on day 7, GnRH on day 9, ET on day 17 (i.e., 7 days after predicted estrus). Embryos were transferred either without freezing (unfrozen) or after freeze-thawing (frozen).
[d]AI versus ET, $P < 0.001$.
[e]AI versus ET-SO and ET-IVF, $P > 0.10$; ET-SO versus ET-IVF, $P < 0.02$.
[f]TET-fresh versus TET-frozen and TAI, $P < 0.01$.
[g]TET-fresh versus TET-vitrified and TAI, $P < 0.05$.

is low metabolic heat production. Therefore, selection for thermotolerance could indirectly lead to selection for reduced feed intake, milk production, or growth. It also is possible to select for specific traits conferring resistance to heat stress (including coat color and the *slick* gene controlling hair length in cattle), but potential advantages of such an approach are diminished by reduced selection for traits of economic importance such as milk production. A better approach may be to select for production in the hot climate itself; data from beef cattle suggest that this approach results in indirect selection for thermotolerance.

PHOTOPERIOD AND REPRODUCTION

Although cattle are not seasonally anestrous animals, photoperiod modifies reproductive processes controlling the establishment of estrous cycles at puberty and during the postpartum period. Photoperiod also may affect fertility. The pineal gland appears to be the mediator for many actions of photoperiod.

Puberty

Two studies indicate that exposure to short photoperiods early in life hasten puberty. In Wisconsin, heifers born in autumn attained puberty sooner than those born in spring, regardless of photoperiodic and temperature environments experienced in the second 6 months of life. Furthermore, puberty was hastened in heifers born in February-March and given a melatonin ear implant at approximately 100 days of age for 5 weeks, to simulate short days. In contrast with the situation existing until 3 to 5 months of age, long photoperiods are the stimulatory photoperiod in older calves. Exposure of calves to artificial days of 16 to 18 hours of light per day (for a pattern of hours of light–hours of darkness [L:D] of 16L:8D or 18L:6D) reduced age and weight at puberty (i.e., first ovulation) in heifers and increased circulating concentrations of testosterone at 14 to 20 weeks of age in bulls. An example of the magnitude of photoperiodic effects on puberty is shown in Figure 56-6.

The prepubertal period in cattle occurs over a long time span that ranges from 6 to 24 months or longer. Consequently, the influence of season of birth on onset of puberty depends on other genetic and environmental factors that determine the length of the prepubertal period. For example, heifers in Wisconsin born in the spring reached puberty earlier than winter-born heifers if fed a diet high in energy, whereas the opposite was true if heifers were fed a diet low in energy.

The Postpartum Anestrus

In Northern latitudes, the duration of the postpartum anestrus often is shorter for cows calving in the summer or autumn than for cows calving in the winter or spring. The magnitude of the seasonal effect is greater for cows that have other factors that dispose them to a long postpartum anestrus, such as primiparity, suckling, low energy intake, and genetic capability for high milk production. Seasonal differences in the resumption of estrous cycles after calving probably result from variation in many factors, such as nutrition and housing. Photoperiod is one of these inputs, because exposure of suckled beef cows that calved in the winter to 18L:6D reduced the duration of postpartum anestrus. The magnitude of the beneficial effect of supplemental lighting also was greatest for cows with the longest postpartum anestrous periods.

Other Effects of Photoperiod

Several reports indicate that supplemental lighting during the winter increases pregnancy rates. In other reports, long photoperiods increased sperm motility and decreased sperm abnormalities in bulls and reduced interval to uterine involution. Effects of photoperiod extend to *B. indicus*, even though these cattle evolved in an environment with little seasonal variation in day length. Brahman cows subjected in the winter to 14L:8D experienced higher pregnancy rates and reduced frequency of "silent" ovulations, compared with cows exposed to natural photoperiods.

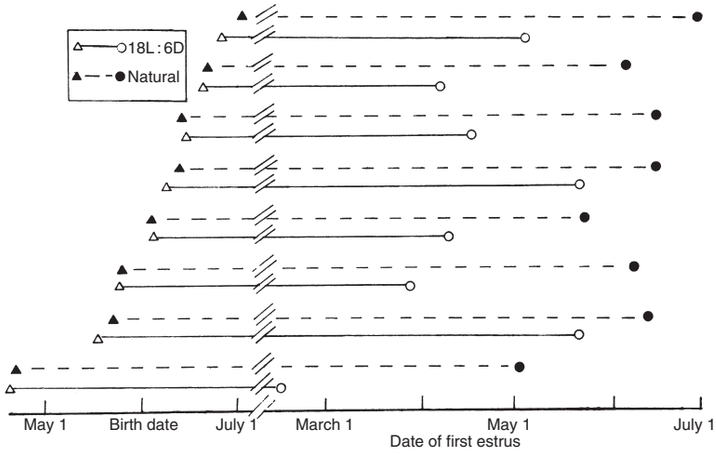

Fig. 56-6 Effect of photoperiod on puberty in heifers reared in Wisconsin. Beginning at 5 months of age, heifers either continued exposure to natural photoperiods or were exposed to a daily pattern of 18 hours of light and 6 hours of darkness (18L:6D). Shown are individual dates of birth (*triangles*) and first estrus (*circles*). (From Hansen Kamwanja LA, Hauser ER: *J Anim Sci* 1983;57:985–992, with permission of *Journal of Animal Science*.)

Mechanism of Action of Photoperiod

In seasonally breeding species, the pineal plays a central role in mediating effects of photoperiod on the hypothalamic-pituitary axis through secretion of the hormone melatonin. As in seasonally breeding species, secretion of melatonin in cattle exhibits a diurnal pattern, with highest concentrations during darkness. The duration of elevated melatonin secretion is proportional to the length of darkness, although the two periods are not identical. Involvement of the pineal in photoperiodic regulation of reproduction in cattle is indicated by the finding that administration of a melatonin implant hastened onset of puberty when administered at approximately 100 days of age and slightly lengthened the postpartum anestrus in beef cows.

Some evidence indicates that photoperiodic modulation of reproduction is exerted at the level of the hypothalamic-pituitary axis. LH secretion early in life was greater for heifers born in September than for those born in March. Also, circulating concentrations of LH and follicle-stimulating hormone (FSH) were greater in ovariectomized heifers exposed to days of increasing length than in those exposed to days of decreasing length. Supplemental lighting in the winter also increased the magnitude of estradiol-induced LH secretion. In contrast with these findings, exposure to 18L:6D had no effect on LH secretion in postpartum cows, prepubertal bulls, or ovariectomized heifers, compared with natural winter photoperiods. Furthermore, long days or afternoon injections of melatonin inhibited LH and FSH secretion in ovariectomized heifers given an estradiol implant.

Secretion of prolactin is markedly increased by photoperiod, but it is unlikely that alterations in secretion of this hormone are responsible for changes in reproductive activity. Exposure to 16L:8D hastened onset of puberty in heifers, even though prolactin secretion became refractory to photic influences. Additionally, treatment of postpartum cows with dopamine agonists to lower prolactin secretion did not affect length of the postpartum anestrus. Effects of photoperiod on reproduction also are not mediated by changes in body weight. Some component of photoperiodic effects may be mediated by metabolic changes, however, because treatment of heifers with growth hormone–releasing hormone reduced beneficial effects of a 16L:8D photoperiod on age at puberty.

Schemes to Improve Reproduction through Manipulation of Photoperiodic Signals

Practical advantages accruing from manipulation of photoperiod are likely to be greater in the postpartum period than in the prepuberium. The magnitude of the influence of photoperiod on age at puberty is small, and earlier puberty does not necessarily lead to earlier establishment of pregnancy. By contrast, seasonal and photoperiodic effects on duration of the postpartum anestrus and calving interval sometimes are large. Supplemental lighting during short days also increases milk yield.

The minimum photoperiod necessary to enhance postpartum reproduction is not known. The only photoperiod that has been tested experimentally for postpartum cows is 18L:6D. A 14L:10D photoperiod, however, was found to enhance other aspects of reproductive function in Brahman cattle and probably is adequate. Work from studies on photic regulation of hormone secretion can be used to estimate some of the other characteristics of supplemental lighting required to affect postpartum reproduction. One economical way to manipulate photoperiod may be through use of "skeleton" lighting, in which animals are exposed to a short period of light during darkness to entrain them to a long photoperiod. Exposure of bulls and cows to 2 hours of light beginning 13 to 20 hours after dawn in animals otherwise receiving 6 hours of light per day increased secretion of prolactin. The intensity of light necessary to suppress melatonin secretion was as little as 400 lux, whereas light levels as low as 200 lux increased prolactin secretion. The spectrum of wavelengths of light that are effective evidently is broad, because supplemental lighting from fluorescent, incandescent, mercury vapor, red, or blue lamps has been shown to increase prolactin secretion.

OTHER STRESSES

As mentioned earlier, the low heritability associated with reproductive traits in cattle constitutes a testament to the

importance of environment on reproductive function. This chapter has emphasized the importance of heat stress and photoperiod, but many other environmental inputs contribute to variation in reproductive function of cattle. A brief review of some of these factors is presented next.

Cold Stress

Having evolved in cold and wet climates, adult cattle of European origin seem to be little affected by cold stress. Exposure to cold increases metabolic rate and energy balance, but the environmental temperatures at which this occurs are very low. Cold stress has the greatest consequence for the newborn calf, which has a poorly developed thermoregulatory system. *B. indicus* calves are particularly susceptible to the development of hypothermia. Exposure to cold stress also can cause scrotal frostbite of bulls, and the resultant edema of the scrotum results in increased scrotal temperature and infertility.

Social Factors

Abundant evidence indicates that interval from calving to first estrus in postpartum beef cows is reduced by the presence of bulls or androgenized cows. It is equivocal, however, whether a similar effect occurs in prepubertal heifers. Also, libido and mating ability of young bulls were not affected by exposure to heifers during rearing. In adults, temporary isolation has been reported to decrease libido of bulls, and maintenance of cows in tie stalls delayed resumption of ovarian activity after calving, as compared with untethered cows. Social status also can affect reproductive function: In one report, interval from calving to conception was shorter in cows experiencing an increase in dominance rank than in cows experiencing a decrease in dominance rank.

The extent to which estrous activity occurs is dependent on social factors. Mounting activity per cow increases if several cows are in estrus together, because cows that are under the influence of estrogen are more likely to mount cows in estrus than are cows under the influence of progesterone. Number of mounts and accuracy of estrus detection also increase if androgenized cows are present in a herd. Some evidence also indicates that mounting activity is stimulated if cows in estrus are unfamiliar to detector animals.

Shipping Stress and Emotional Stress

Data are equivocal regarding whether reproductive activity is susceptible to stress associated with rough handling or fear. For example, characteristics of estrus in ovariectomized heifers and cows treated with estradiol cypionate were not affected by a variety of stresses including acute starvation and being chased by a dog, soaked with water, or worked in a chute while receiving a drench and insecticide spray. In addition, repeated handling stress during the follicular phase of the estrous cycle had no effect on estrous behavior, although two of seven stressed cows did not undergo detectable preovulatory surges of LH. In another study, however, the stress of transportation reduced superovulation response. In still another report, transportation reduced induction of LH surges induced by estradiol benzoate treatment in noncycling cows (but not in cyclic cows).

Confinement

Confinement represents the most recent example of large-scale, manmade alterations in the environment in which cattle exist. It is reasonable to assume that, as with any environmental change, confinement has increased the stress imposed on cattle. Very little is known about the effects of confinement on reproduction, even though increasing numbers of cattle are managed in such environments. Concrete flooring reduces the expression of estrous intensity, and lameness, which is one of the consequences of housing cattle on inadequate flooring, is associated with lower pregnancy rates per insemination. Other effects of footing surface (including the effects of mud) on reproduction are unknown.

Flies often are abundant in confinement housing, and some data indicate that exposure to flies exacerbates problems of heat stress. Little is known about the potential for exposure to environmental pollutants associated with confinement. This is a potentially important topic because manure gases have been reported to delay onset of puberty in gilts.

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NOTE: For more information on the primary references supporting the statements in this chapter, the reader is referred to the first edition of this volume, as well as to references 8 to 12.

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CHAPTER 57

Effects of Nutrition on Reproduction in Dairy Cattle

JAMES N. SPAIN, MATTHEW C. LUCY, and DAVID K. HARDIN

Walton, as cited by Coppock,[1] predicted that individual cow yields of more than 31,000 kg of milk per year were attainable, with the potential for herd averages of 16,000 kg of milk per year. Breed averages for milk yield increased 40% to 60% for first-lactation cows between 1960 and 1988.[2] With continued genetic progress and improvements in management systems, Walton's predicted levels of milk production are reasonable and achievable goals.

As milk production increases, fertility of lactating dairy cattle, as a general rule, decreases. A survey of commercial herds in North Carolina showed a decrease in conception rate from 52% to 32% in a comparison of the lowest-producing (n = 425) and the highest-producing (n = 53) herds.[3] Days open and days to first service were similar for both groups. More recently, Washburn and associates[4] summarized Dairy Herd Improvement Association (DHIA) data from 10 states in the southeastern United States. In the period 1976 to 1999, annual milk yield per cow increased 1800 kg, while days open increased from 112 to 152 during the same time period. The herds also experienced an increase in services per conception (from a mean of 1.9 to 2.9). Lucy[5] completed an extensive review and suggested that high levels of milk production are not the sole cause of reduced reproductive performance. Indeed, overall management including housing, health, and nutrition practices also affects fertility of lactating dairy cattle. These findings emphasize the importance of management in maintaining reproductive efficiency as herds achieve higher levels of milk production.

Nutritional status and nutritional management are essential components of overall herd management. The nutritional status of the herd also affects the reproductive soundness and efficiency of the herd. Bauman and Currie[6] described the hierarchy of nutrient utilization by the lactating dairy cow. Within this ranking, reproduction lists below maintenance (necessary for survival), production (milk produced for the young), and growth. Royal and co-workers[7] summarized strategies to improve fertility in dairy cattle, including genetic, endocrine, and nutritional approaches. Ferguson[8] categorized nutritionally associated reproductive problems as primary or secondary. **Primary** conditions of malnutrition affecting reproduction include clinical deficiency and excessive intake of a particular nutrient. **Secondary** conditions comprise those in which other constraints result in a nutritional imbalance. Therefore, a suboptimal plane of nutrition can result in impaired reproductive performance. This observation holds true for both the developing replacement dairy heifer and the lactating dairy cow.

This chapter reviews the effects of nutrition on the reproductive biology of the dairy cow, with regard to both onset of puberty and lactation.

PUBERTY

Gill and Allaire[9] analyzed DHIA records from Ohio and concluded that the optimal age for Holstein heifers at first calving for total lifetime production was between 23 and 24 months. More recent research demonstrated that heifers calving early (22.9 months of age) produced more milk than those calving at an older age (26.5 months).[10] To achieve an average age at first calving of 24 months, heifers must reach puberty by 8 to 9 months of age. This timing would allow heifers to establish cyclicity before

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the breeding period. Establishing cyclicity before breeding has been shown to improve fertility. For example, the conception rate for heifers at pubertal estrus was 21% lower than at their third estrus.[11] An excellent plane of nutrition is critical for heifers to reach puberty and first calving at 9 months and 24 months of age, respectively. Lammers and colleagues reported that heifers needed to gain 820 g/day (average daily gain from birth to calving) to achieve body weight and age targets at first calving.[12]

A number of factors that include body weight, body size, plane of nutrition, breed, and season influence age at onset of puberty. Social environment and phase of the moon also have been reported to alter onset of puberty.[13] Nutrition, because it influences body weight and size, contributes to the onset of puberty. The age at which a heifer begins regular estrous cycles also is correlated with the rate of gain from birth to puberty. Bortone and colleagues[14] reported that heifers fed 115% of National Research Council (NRC) recommendations from 3 to 12 months of age were 22 days younger at the onset of puberty than control animals fed 100% of NRC levels. The correlation between gains in body weight and age at puberty indicates that an increased growth rate in heifers reduces the age at puberty.[15,16] The study of Bortone and colleagues[14] also found that the body weight of heifers at the onset of puberty was the same for faster-growing heifers as for controls. These data indicate that body weight can be used to predict the onset of puberty and establish rate of gain.

Feeding heifers to reach target weight at a selected age for a given genotype is a practical management tool to ensure high potential fertility. Nutrient intake that supports growth and development is essential to achieve a target weight by 9 months of age. Nutrient intake is controlled by growth rate, fiber and energy concentration (or density) of the diet, and the dry matter content of the diet. Tomlinson and associates,[17] using prepubertal and pubertal heifers, found maximal dry matter intake at a neutral detergent fiber level of 41% and an acid detergent fiber level of 20% of the dietary dry matter. Quigley and co-workers[18] reported that intake could be limited by diets containing greater than 23% acid detergent fiber. Intake with high-fiber diets may be limited by the physical constrictions of rumen fill and distention. A decreased rate of fermentation and rate of passage of high-fiber diets also can limit dry matter intake.[19] Nutrient density and fiber content of the diet should be evaluated when animals do not reach the production goals of puberty, breeding, and first calving.

The ration should provide adequate amounts of required nutrients in a form that stimulates intake. Excessive grain (or starch) supplementation should be avoided. Excessive starch feeding can predispose the animal to ruminal acidosis and laminitis. This disorder can limit performance of the animal by decreasing eating time and obviously will slow the rate of gain. Fibrous feeds have been used as substitutes for cereal grains. These feedstuffs can support optimal growth while minimizing the potential effects of supplementation with high-starch grain mixes.

Feed additives also have been used in the diet of developing heifers. Sodium bicarbonate and yeast have been studied for use in the diet of young calves. Observed effects on intake, ruminal fermentation, and health have been variable and nonconclusive.[20] The use of ionophores generally improves growth rates, as well as feed efficiency, of ruminants. Two compounds commonly used are lasalocid and monensin. Feeding ionophores has been shown to decrease age at first breeding and calving, primarily because the growth rate to adequate body size and weight is accelerated. It has been hypothesized that monensin decreases age at puberty by a mechanism independent of weight gain.[21] Estradiol implants also have been found to improve feed efficiency in prepubertal Holstein heifers.[11]

Plane of nutrition also can influence the onset of puberty. Plane of nutrition can inhibit the onset of puberty in heifers at an adequate body weight, size, and age. Gonzalez-Padilla and associates[22] demonstrated that 14-month-old heifers could be maintained in a prepubertal state and then suddenly made to ovulate by increasing nutrient intake. It generally is accepted that the physiologic cause of the delay is the result of inhibition in pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus and the subsequent release of luteinizing hormone (LH) from the pituitary gland. It also has been demonstrated that GnRH secretion is extremely sensitive to the nutritional status of the animal. Furthermore, insulin may play a role for energy-yielding nutrients, as it binds receptors in the region of the brain thought to regulate GnRH secretion.[23] Conditions such as disease and parasitism can influence nutritional status by altering intake or absorption of nutrients. Bumgarner and coworkers[24] reported that calves treated with anthelmintics in both mid-June and mid-July had a reduction in fecal oocyte counts and increased daily gain. Numerous other studies demonstrated improved weight gains from the use of anthelmintics.[25] Respiratory disease that results in severe lung damage can also result in poor performance of growing dairy heifers. A sudden onset of disease can result in a transient period of anestrus.

LACTATING COW

As discussed earlier, Bauman and Currie[6] described the need of lactating cows to prioritize nutrient utilization. In general, energy is the nutrient of primary concern relative to supporting maintenance, lactation, and reproduction. Imbalances or deficiencies of protein, minerals, or vitamins also have been reported to adversely affect the reproductive function of lactating dairy cows. Nutritional imbalances or deficiencies are most important during the period immediately preceding parturition through conception. This section focuses on the influence of nutritional status at critical points throughout lactation.

The nutritional balance of a dairy cow during the periparturient period can have a tremendous influence on fertility and reproductive efficiency. During the final 30 days of gestation, lactation is initiated, with production of colostrum and final growth of the fetus. At day 270 of gestation, the uterus and fetus require greater than 1600 kcal per day.[26] Concurrent with increased nutrient

requirements, the prepartal cow experiences a marked decrease in dry matter intake. Severe anorexia or an imbalance of nutrient intake can predispose the animal to a number of metabolic diseases that constitute the **parturition disease complex.**[27] These diseases are not independent but can increase the risk of subsequent problems. For example, Curtis and coworkers[28] reported that cows with hypocalcemia were at increased risk of suffering dystocia, retained fetal membranes, and ketosis. Dystocia and retained fetal membranes are predisposing factors for postparturient uterine disease. Markusfeld[27] documented that cows experiencing postparturient uterine disease had a decreased milk yield for the lactation and increased number of days open. Retained fetal membranes also have been influenced by selenium and vitamin E supplementation. Cows that experienced retained fetal membranes may have had a lower plane of nutrition. Disrupted intake accompanied by disrupted energy metabolism can result in an increased level of circulating ketone bodies. Miettinen[29] reported that cows with higher concentrations of circulating ketone bodies had more days to first service, decreased first service conception rates, and more days open. More recently, cows with serum beta-hydroxyl butyrate concentrations of 1100 μmol/L in weeks 1 and 2 of lactation were found to have increased risk of conception failure.[30] These studies make a significant statement regarding the importance of periparturient health and metabolism on fertility and reproductive efficiency during subsequent lactation. The primary modulator of reproductive function appears to be energy intake and the energy status of the animal.

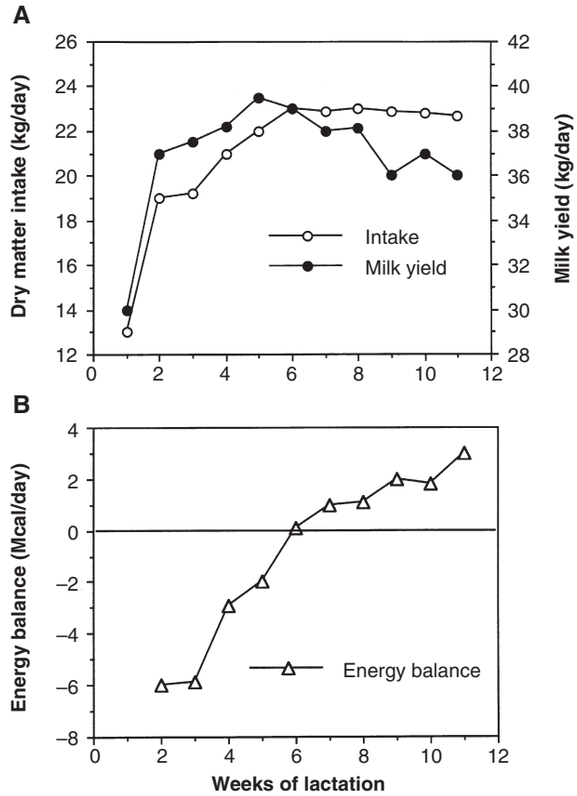

Fig. 57-1 Average daily dry matter intake and daily fat corrected milk yield **(A)** and daily energy balance **(B)** for lactating Holstein cows ($N = 17$). (From Lucy MC, Staples CR, Thatcher WW, et al: Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. *Anim Prod* 1992;54:323.)

Energy

Energy or energy balance is considered the primary nutrient relative to nutritional modulation of reproductive function of the dairy cow. The energy balance of the animal is most simply expressed by the equation

Energy balance
= energy intake (milk energy secreted
maintenance energy)

With initiation of lactation, the acceleration in milk production exceeds the increases in dry matter intake. Kertz and colleagues[31] showed that dry matter intake increased gradually, to peak at week 10 after calving. Lactating cows consume inadequate dietary energy to meet peak milk production. As a result, early-lactation cows experience a negative energy balance (Fig. 57-1). On the basis of energy calculations, cows in early lactation can experience as much as a 20-Mcal deficit of net energy. Cows compensate during this delay in energy intake by mobilizing stored body energy. For the early postpartum cow, this energy is generated primarily from stored adipose tissue. One kilogram of body fat contains 6 Mcal of stored energy.[31] Therefore, early-postpartum dairy cows must rely on oxidation of body fat for energy to compensate for inadequate energy intake.

The nutritional challenge of negative energy balance is significant and can adversely affect the reproductive performance of the dairy cow (Fig. 57-2). A similar effect is recognized in most species. This response by the animal may represent a mechanism to conserve energy during periods of starvation. This same mechanism, however, allows the animal to synchronize reproductive events with periods of positive nutritional status. The timing of these nutritional challenges is an important consideration in management, in view of the need to have high fertility and conception rate during early lactation. The inhibitory factors that prevent pregnancy are only partially understood. Negative energy balance can increase the period of postpartum anestrus and decrease fertility at first and subsequent inseminations, depending on the severity of the imbalance.

Cows should experience the first postpartum ovulation by 30 days after calving. Cows are not inseminated at this time because of incomplete involution of the uterine tissues. It is important, however, to minimize the length of postpartum anestrus for two reasons. First, cows with long intervals to first ovulation will be anestrous during the breeding period. Conception will therefore not occur because the cow does not express estrus and does not ovulate. Second, a shorter time to estrus will enable the cow to have multiple ovulations before the first insemination. The estrous cycle has a cleansing effect on the

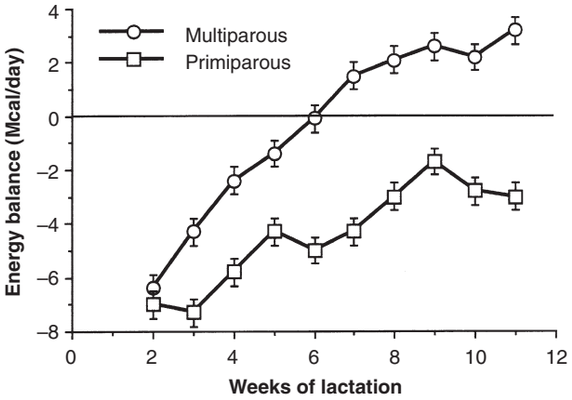

Fig. 57-2 Average daily energy balance for multiparous (n = 35) and primiparous (n = 15) Holstein cows. (From Lucy MC, Staples CR, Thatcher WW, et al: Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. *Anim Prod* 1992;54:323.)

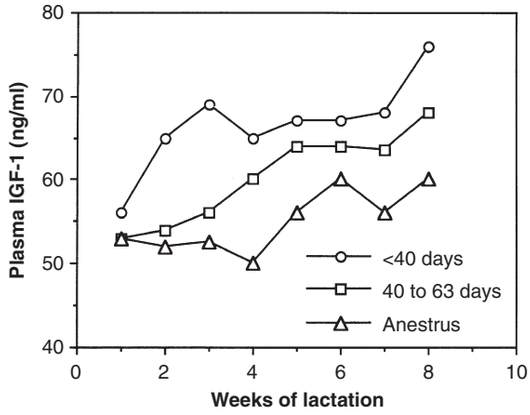

Fig. 57-3 Concentrations of insulin-like growth factor-I (IGF-I) in Holstein cows with different intervals to first ovulation. Interval to first ovulation was less than 40 days (n = 25), 40 to 63 days (n = 14), or more than 63 days (anestrus, n = 15). (From Thatcher WW, de la Sota RL, Schmitt EJ, et al: *Reprod Fertil Dev* 1996;8:203.)

uterus, with increased uterine contractility and relaxation of the cervix. Therefore, as shown in a large study conducted in Florida,[32] cows with prebreeding estrus had higher first-service conception rates (47%) compared with cows with no prebreeding estrus (34%).

To achieve the maximum number of estrous cycles before breeding, cows must ovulate during the early postpartum period (<21 days in milk). Research has shown that cows will ovulate approximately 10 days after reaching the lowest point of negative energy balance, the nadir.[33] Growth of follicles and ovulation are dependent on the pulsatile secretion of LH.[34] In cattle in severe negative energy balance, the secretion of LH is inhibited.[35] Disrupted or decreased LH secretion slows the growth and development of the follicle, which delays ovulation.[36] Insulin and insulin-like growth factor-I (IGF-I) also are required for normal follicular growth and ovulation. Cows in negative energy balance have reduced levels of IGF-I. This is an important relationship, given that IGF-I amplifies the effects of LH on the ovary through potentiating the signaling mechanism for LH.[37] Therefore, the actions of LH are decreased in cows in negative energy balance because lower IGF-I leads to reduced effectiveness of LH. Consequently, follicles in cows with extremely low IGF-I do not develop normally, and ovulation is delayed because LH is less active. This effect was demonstrated in a study by Thatcher and co-workers[38] (Fig. 57-3). Cows that had first ovulation before 40 days post partum had the highest concentrations of plasma IGF-I.

In accordance with these findings, the interval to first ovulation is controlled primarily by energy balance.[39] One of the most important factors that determine when the nadir will occur is postpartum feed intake. Postpartum dairy cattle that have the greatest increase in dry matter intake after calving are most likely to pass their energy nadir early and have the shortest interval to first ovulation (Fig. 57-4). In a study in Illinois, cows that consumed the most feed had the shortest interval to first ovulation.[40] Anestrous cows (>42-day interval to first

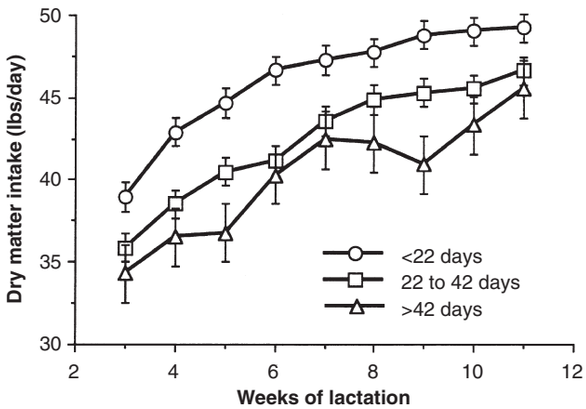

Fig. 57-4 Average daily dry matter intake for Holstein cows with different intervals to first ovulation. Interval to first ovulation was either less than 22 days (n = 17), 22 to 42 days (n = 18), or more than 42 days (n = 5). (From Lucy MC, Staples CR, Thatcher WW, et al: Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. *Anim Prod* 1992;54:323.)

ovulation) had the lowest dry matter intake. On the basis of these results, high-quality diets must be fed during the early postpartum period. In addition, postpartum metabolic disorders must be prevented so that cows can resume the maximal rate of recovery of dry matter intake after calving. These conditions not only maximize milk production but also improve feed intake and decrease the interval to first ovulation.

In addition to decreased interval to first ovulation, improved energy balance during early lactation can decrease the incidence of other ovarian diseases. For example, cystic ovaries are most prevalent in high-producing dairy cows that are in negative energy balance. Although this phenomenon is not fully understood, a

common link between this disease and negative energy balance is the abnormal secretion of LH.[41] Cystic ovaries are associated with increased LH secretion but an inhibition of the preovulatory LH surge. The cumulative effect of these conditions is development of a large cystic follicle that fails to ovulate in the absence of the ovulatory surge of LH. This relationship further strengthens the relationship among energy balance, LH, and early postpartum reproductive performance of the dairy cow.

Energy status at the time of breeding also can affect reproduction. Several reviews summarized by Weaver[42] conclude that cows that lose weight at the time of breeding have lower fertility. This decrease in fertility may be related to decreased progesterone secretion by the corpus luteum. Progesterone concentrations before and after insemination have been positively correlated with fertility.[43] The exact relationship between low progesterone and fertility has not been described; however, several reports have related low progesterone concentrations in cows that are losing weight.[44,45] In addition, cows in good body condition (body condition score [BCS] of 2 or 3 on a scale of 1 to 5) had better conception rates when receiving a transplanted embryo than did thin cows.[46] This study demonstrates the importance of uterine function in fertility and the possible role of progesterone in fertility in thin cows. Inadequate uterine function may be related to lower progesterone concentrations in cows in poor body condition. Furthermore, progesterone release in response to LH is severely diminished in the absence of IGF-I.

Thus, the effects of negative energy balance can be multiple and related. First, severe negative energy balance at calving can predispose the animal to increased days to first ovulation. This relationship appears to be hormonally modulated by insulin, LH, IGF-I, and progesterone. Insulin, LH, and IGF-I appear to influence the function of the ovary. In addition, the decreased pulsatility of LH decreases progesterone. Decreased progesterone has been shown to decrease fertility, possibly owing to poor uterine function. Therefore, energy balance or feed intake does affect reproductive performance of dairy cows.

Protein

Protein nutrition of the lactating dairy cow is confounded by energy balance. These animals have very limited labile protein stores, so diet is the primary source of nitrogen. Because of extensive pregastric fermentation by ruminal microorganisms, protein presented for absorption in the small intestine is altered considerably relative to the dietary protein actually consumed. Dietary protein and protein digestion have been categorized by location and rate of digestion of the protein.[47] As a result of the presence of rumen microflora, ruminants can use nonprotein nitrogen sources to produce a high-quality microbial protein for digestion in the small intestine. Dietary crude protein (CP) that is fermented by rumen microflora is defined as **rumen-degradable protein** (RDP). This protein is degraded to ammonia, single amino acids, and peptides. Ammonia produced in excess of that which the microbial population can utilize is absorbed from the rumen and transported in the circulatory system. A major portion is converted to urea in the liver. Feeding diets high in total CP or diets containing an excess of RDP or soluble CP can predispose lactating cows to elevated blood urea nitrogen (BUN) levels. Butler[48] completed an extensive review on the effects of protein nutrition on reproductive function and suggested several potential modes of action, including altered uterine environment, altered endocrine profiles, and indirect effects from altered energy partitioning during periods of negative energy balance.

A number of studies have been conducted to evaluate the relationship between dietary protein level and source with BUN level and conception rates in dairy cattle. Ferguson and Chalupa[49] summarized published data and used logistic regression to generate a predictive model. The model estimated the effects of RDP and undegraded intake protein on fertility in dairy cows. These workers concluded that a serum urea nitrogen level of greater than 20 mg/dL resulted in a lower conception rate. Hutjens and Jordan[50] summarized eight studies that evaluated BUN and conception rate. Cows fed 19% to 21% CP diets had higher BUN (21.3 versus 13.8 mg/dL) and lower conception rate (62% versus 48%) compared with cows fed diets containing 15% to 16% CP. Guo and associates[51] studied the relationship between milk urea nitrogen (MUN) using DHIA data representing 10,000 cows in more than 700 herds. These scientists reported that MUN was related to decreased fertility within the herd, but the effect was inconsistent across herds. What has not been clearly defined are the mechanisms involved in this relationship. Elevated blood levels of ammonia or urea or both could alter secretions produced in the reproductive tract itself and affect viability of the ova, sperm, or embryo. In addition, the hormonal balance required for normal function also may be involved.[48] These two areas have been the focus of much published research.

A series of studies has shown increased urea nitrogen levels in blood and in oviductal and vaginal fluids.[52] Williams and coworkers[53] also found that high-protein diets increased the urea nitrogen content of uterine secretions but found no differences in fertility or in vitro embryo development. Jordan and coworkers[54] found that high-CP diets changed the concentrations of magnesium, potassium, phosphorus, and zinc in uterine secretions. Overall, high-protein diets increase the nitrogen content of uterine secretions, in addition to shifting mineral composition; however, a direct effect of these changes in utero on fertility and embryo viability is less clear. Blanchard and others[55] evaluated the effects of a high-RDP diet on fertilization and embryo quality. Cows fed high-RDP diets had similar numbers of fertilized, unfertilized, transferable, and nontransferable ova compared with cows fed low-RDP diets. Elrod and Butler[56] proposed that the products of excessive protein degradation in the rumen decreased uterine pH, which may reduce fertility. Garcia-Bojalil and associates,[57] however, reported no difference in ovarian follicle development or number and quality of recovered embryos due to protein level (12.3% versus 27.4% CP). These data suggest that other mechanisms are involved.

Altered hormonal balance caused by elevated BUN levels also may decrease fertility and reproductive

performance. Visek[58] reviewed the effects of ammonia on metabolic hormones and reproduction. Excessive ammonia or chronically elevated ammonia may alter hormonal status and thus performance of the animal. The metabolic signals have not been established, but intermediates of the urea cycle such as arginine may potentially cause shifts in insulin and alter glucose metabolism. Fernandez and associates[59] showed that subclinical ammonia toxicity decreased insulin concentrations in the plasma of steers. Decreased insulin may suggest a disruption in fertility similar to that described earlier for periods of negative energy balance and low circulating insulin concentrations. The shift in hormonal balance also was associated with reduced glucose utilization by insulin-sensitive extrahepatic tissue. Therefore, excess ammonia absorbed from the rumen of cows fed high-RDP or soluble-CP diets could down-regulate a hormonal or metabolic signal to the ovary.

In addition to metabolic hormones, studies also have evaluated the effects of reproductive hormones. Two studies[60,61] showed that the amplitude of the LH peak was higher for cows fed high-protein diets. Progesterone was more variable, with one study showing lower progesterone[60] and the other showing no differences.[61] These studies did not establish an endocrine mechanism through which high-CP diets might alter reproduction.

A confounding factor is the milk production response often observed with high-protein diets. Carroll and associates[62] fed cows diets containing 13% or 20% CP. Cows fed a high-CP diet produced more milk (2.7 kg) during peak lactation; however, the reproductive parameters monitored exhibited few differences due to diet. Howard and colleagues[63] reported similar results in a larger study conducted in Oklahoma. No differences were observed in conception rate or days open due to level of protein in the diet. In both studies, reproductive efficiency was excellent, as indicated by fewer than 1.8 services per conception and average days open less than 85. Therefore, excellent reproductive management of high-producing cows can maintain reproductive efficiency.

Minerals and Vitamins

Several excellent reviews on the effects of mineral nutrition and status on reproductive function of dairy cattle are available.[64–68] These surveys discuss the impact of mineral and vitamin status on reproductive function and performance.

Calcium as an individual nutrient has been associated with poor reproduction when severely deficient. Calcium, the relationship of calcium to phosphorus, and the balance of calcium with vitamin D also have been linked to altered reproductive performance. Ward and coworkers[69] reported that cows fed high-calcium and high–vitamin D diets ante partum had more rapid uterine involution, fewer days to first service, and fewer days open. Hignett and Hignett[70] often are cited for their work with the calcium-phosphorus balance and vitamin D to support normal reproduction. From their results, a minimum calcium-to-phosphorus ratio of 1.5:1 and a minimum daily P intake of 30 g were suggested. As Wiess[68] notes, current NRC recommendations agree with these data, but data relating recommended levels to fertility and reproductive performance have not been reported.

Macromineral and vitamin nutrition also can indirectly affect reproductive health and performance. Curtis and associates[28] described the relationships between hypocalcemia and periparturient disorders. Parturient hypocalcemia significantly increased the odds ratio for dystocia and retained placenta. These investigators concluded that the loss of muscle tone associated with hypocalcemia could reduce normal uterine function. Early research found that feeding low-calcium diets prevented parturient paresis.[71] Practical dry cow diets often are low in calcium, but cattle suffer from hypocalcemia. Block[72] reported the advantage of shifting the dietary cation-anion balance on calcium status. Manipulation of the anionic content of the diet has been used to lower the incidence of hypocalcemia and clinical milk fever. As discussed earlier, decreasing these metabolic disorders probably would improve reproduction. Morrow[73] reported that dairy heifers suffering from phosphorus deficiency had high rates of infertility as measured by services per conception. Increasing phosphorus in the diet returned blood levels to normal, with improved fertility.

Several trace minerals have been found to be important for reproduction.[65] Selenium and vitamin E are the two most often considered for change in herds suffering from reproduction problems; however, other microminerals also have been recognized to be important. Ingraham and colleagues[74] found that copper supplementation increased fertility when combined with increased phosphorus. Manganese also has been shown to influence reproduction. Wilson[75] improved the conception rate among cows in 12 cooperating dairies by feeding supplemental manganese. Zinc is recognized as an essential nutrient required for normal growth. Zinc also has been identified as a component of or cofactor for more than 200 proteins and enzymes. Although zinc's role in metabolism has been defined, including the growth and repair of normal epithelial tissue, the role in reproduction is not fully understood. Two reviewers have examined the importance of zinc for testosterone biosynthesis and spermatogenesis.[65,76] Little work has been completed for the mature cow. Apgar[77] reviewed zinc's influence on reproduction for all species. Zinc deficiency increased the length of labor and bleeding time in the rat. This relationship also has been reported for the ewe. In all species, the possible influence of zinc on endocrine changes has not yet been adequately described.

Selenium and vitamin E have been the two trace minerals most extensively researched. Trinder and coworkers[78] reported that supplemental selenium and vitamin E 30 days ante partum reduced the incidence of retained placenta. Supplemental selenium and vitamin E also reduced delay in delivery of the fetal membranes. Similar reports have been published from other laboratories[79,80]; others, however, have reported no benefits of selenium or vitamin E.[81,82] Segerson and associates[83] noted that only marginally deficient cows responded and that cows with adequate selenium or those with severe deficiency did not respond to the diet. In summary, selenium and vitamin E are necessary in the diet of the dry cow

and the milking cow to maintain normal reproductive function. Supplementation above recommendations or in severely deficient animals will not provide reproductive benefits.

Beta-carotene (carotene) also has been investigated as a nutrient having special requirements for reproduction. Hemken and Bremel[84] reviewed this topic in 1982 and concluded that adequate evidence did not exist to support the establishment of a requirement for carotene above that for vitamin A. Chew and co-workers[85] reported high levels of carotene in the blood, corpus luteum, and follicular fluid, but effects on ovarian functions were not described. Additional studies also have failed to establish a requirement for carotene.[86–89]

IMPLICATIONS FOR MANAGEMENT

Research has established that the nutritional status of the animal alters the reproductive function of the animal. In fact, the current question is how and to what level nutrition alters the reproductive function of the high-producing dairy cow. Poor nutrition can result from inadequate nutrient density in the diet, inadequate amounts of the diet fed, and insufficient access to the diet. Prepartum metabolic disorders have a carryover effect that can impede reproductive function because of the residual effects of severe negative energy balance. This phenomenon emphasizes the importance of nutritional management, as well as overall management of the herd. This aspect of farm management will become increasingly critical as the genetic base of the dairy cow population continues to improve. As noted by Norman and Powell,[2] tremendous potential exists in the current gene pool of dairy cattle. The long-range challenge will be maximization of this genetic potential to its fullest extent.

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CHAPTER 58

Effects of Nutrition on Reproductive Performance of Beef Cattle

WILLIAM S. SWECKER, Jr., and RAMANATHAN KASIMANICKAM

"If you feed them, you can breed them. If you don't, you won't."

Bill McDonald, seventh-generation beef producer, Montgomery County, Virginia

The relationship between nutrition and reproduction is bidirectional—that is, reproductive status alters nutrient requirements, but the nutrients assimilated by the cow also alter reproductive function. A beef cow must conceive, carry a fetus to term, give birth, and wean a calf in a 12-month period; thus, she is either pregnant or lactating at any given time. The fetus becomes a high priority for nutrient repartitioning in the dam, which can increase nutrient requirements by 75% at the end of pregnancy to support a weight gain of up to 70 kg for the fetus, the placenta, and fetal fluids.[1] Likewise, nutrients are repartitioned again at calving to support lactation, which can increase requirements by 50% to 100% above maintenance.[2] Hormonal changes associated with pregnancy and lactation aid in the prioritization of nutrients; however, a negative energy balance during early lactation modifies the signals that initiate ovarian cyclic activity.

The beef cow utilizes tissue energy, which results in loss of body condition, to counter the negative energy balance of early lactation. The challenge to meet nutrient requirements becomes more difficult with the addition of environmental influences such as cold, rain, heat, and mud. This chapter reviews the requirements of beef cattle relative to their reproductive status and defines abnormalities of reproduction and related nutrient risk factors in beef cows. Nutrition and reproduction in the bull are discussed briefly.

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NUTRITION AND FEMALE REPRODUCTION

Nutrient Requirements of the Beef Cow

Abnormalities in reproductive function may not be detected by the producer or the veterinarian until weeks or months after the causative insult, which may be infectious, toxic, or nutritional. The nutrient demands of the cow relative to the feed supply available can be evaluated historically or prospectively. The nutritional requirements of beef cows vary with their reproductive status[3]:

- *Calving to conception* (82 days): Reproductive functions during this period include uterine involution, resumption of normal estrous cycles, and lactation. Nutrient requirements are greatest during this period, and in most instances the cow must utilize tissue energy to meet these requirements because she cannot consume enough nutrients. Clinically, this is represented by loss of body condition.
- *Pregnant and lactating* (123 days): The primary demand for nutrients during this stage is lactation, and cows should gain weight during this period. Increased nutrient intake supports lactation, which also results in calf weight gain.
- *Midgestation* (140 days): Nutritional requirements are lowest, and cows can be maintained on low-quality feeds during this period. This is an excellent time to increase the body weight of thin cows.
- *Precalving* (50 days): Nutrient requirements rapidly increase during this phase, and body condition should be maintained, in addition to the weight gain caused by an increase in the weight of fetal tissues, placenta, and uterine fluid. Inadequate nutrition, especially during this period, can result in decreased calf survival, lower milk production and slower calf growth, and delayed resumption of ovarian activity.

Energy and protein requirements during each of these four periods are listed in Table 58-1.

Table **58-1**

Protein and Energy Requirements for an 1100-lb Beef Cow during the Reproductive Cycle

Reproductive Period	Net Energy (Mcal/day)	Protein (kg/day)
Calving to conception (85 days)	14.9	1.05
Conception to weaning (125 days)	12.2	0.86
Weaning to late gestation (110 days)	9.2	0.64
Late gestation (50 days)	10.3	0.73

Data from Corah LR: Nutrition of beef cows for optimizing reproductive efficiency. *Compend Contin Educ Pract Vet* 1988;10:659; and Corah LR: Body condition: an indicator of the nutritional status. *Agric Pract* 1989;10:25.

Abnormalities in Female Reproduction

Veterinarians or producers commonly recognize reproductive abnormalities when cows do not calve or when a large percentage of cows are found to be open when examined for pregnancy. The nutritional events that result in failure of cows to become pregnant may have occurred 4 to 18 months previously. Although overlap among diagnostic groups will be inevitable, the practitioner can attempt to categorize the nonpregnant cows as those that failed to have normal estrous cycles, those that failed to conceive, or those that conceived but did not remain pregnant; each category suggests different causes. A thorough review of the diagnosis of nutritional infertility has been published.[4]

Failure to Cycle: Heifers

Most production systems require that heifers calve at 23 to 24 months of age. Heifers that calve early in the calving season wean more and heavier calves during their lives.[5] Heifers, therefore, should reach puberty at 12 to 13 months of age to allow one or two estrous cycles before breeding at 14 months. Age at puberty is influenced by breed, by season, and by plane of nutrition.[5,6] Growth rates during the preweaning and postweaning periods are inversely related to age at puberty, and the postweaning growth rate is associated with plane of nutrition.[5] The onset of puberty appears to be associated with increased frequency of luteinizing hormone (LH) secretion. Increased pulsatile LH secretion is associated with increased energy intake, whereas reduced energy intake suppresses LH secretion in heifers.[7] Delayed puberty also is associated with low concentrations of insulin-like growth factor-I (IGF-I). Proliferation of steroidogenic capacity of thecal[8] and or granulosa cells[9] is associated with decreased concentration of IGF-I. Recent research has focused on leptin as a regulator for reproductive function and particularly as a mediator for nutritional cues. Short-term fasting of peripubertal heifers decreased leptin gene expression, and circulating leptin concentrations were coincident with reductions in circulating concentrations of insulin and IGF-I and in LH pulse frequency, resulting in delayed puberty.[10] Conversely, serum leptin concentration was positively correlated with changes in increasing body weight in prepubertal heifers.[11] Addition of ionophores to the ration increases growth rate but also tends to decrease age at onset of puberty, independent of weight.[12,13]

Historical recommendations have been that heifers should be fed to reach a target weight of 65% to 70% of their mature weight at breeding. Brahma-cross heifers fed to a target weight of 318 kg showed estrus earlier and had increased rates of pregnancy after 20 days of breeding (39% versus 9%) and at the end of the breeding season (82% versus 66%) compared with heifers fed to a target weight of 272 kg.[14] Conversely, spring-born, weaned beef heifers (213 kg) were fed over winter to achieve either 55% or 60% of mature weight at breeding. Heifers fed for 60% mature weight were heavier at breeding (313 versus 289 kg) and had increased condition scores (6.0 versus 5.6), and more were cycling before the breeding season (85% versus 74%). Pregnancy rates at the end of the

45-day breeding season, however, were similar between groups (88% versus 92%) The heifers were then followed through three calvings; production did not differ between groups except for higher 205-day adjusted weaning weights after the second calving for the heifers fed to achieve 55% of mature weight at breeding.[15]

Decreased Reproductive Rates in Mature Cows

Breed, suckling stimulus, and plane of nutrition affect return to estrus in postpartum cows.[16] Body condition score (BCS) at calving and nutrient supply during the early postpartum period affect the return of ovarian cyclic activity and subsequent pregnancy rates (Table 58-2).

Alterations in plane of nutrition and BCS alter hormonal status. For example, pulse frequency of LH was increased 3 weeks post partum in cows that calved in moderate body condition compared with cows that calved in poor body condition.[17] Postpartum LH secretion is less responsive to dietary protein. LH release at 20, 40, and 60 days after calving did not differ between cows fed adequate crude protein before calving (0.96 kg/day) and cows fed restricted crude protein (0.32 kg/day) starting at 90, 60, and 30 days before calving.[18]

Several studies have shown that body condition at calving alters the time between calving and conception and pregnancy rates. Pregnancy rates for cows with BCS of 4 and 5 at calving were lower than pregnancy rates for cows with BCS of 6 and 7 at calving (64.9% and 71.4% versus 87.0% and 90.7%, respectively).[19] In a similar study, cows with BCS of 4 at calving had a pregnancy rate of 50%, whereas cows that calved with BCS of 5, 6, and 7 had pregnancy rates of 81%, 88%, and 90%, respectively.[20] BCS at calving also affects calving interval. Cows with BCS greater than 5 had 10 to 18 fewer days open than cows with BCS of 4.[19] Investigators using a 5-point condition score scale demonstrated a decrease in calving interval of 11.2 days for each unit increase in body condition at calving.[21] This is approximately equivalent to a change of 5 to 6 days in calving interval for a unit change in condition score when a 9-point scale is used. Cows with BCS greater than 5 displayed estrus 12 days earlier after calving and had 14 fewer days open than cows calving with BCS less than 4.[18] A cubic relationship between condition score at calving and pregnancy rate has been described: The effect of a 1-unit change in BCS on pregnancy rate was found to be greater for cows with BCS between 4 and 6 than for fatter or thinner cows.[23]

One study demonstrated that BCS at the beginning of the breeding season influenced pregnancy rate and calving interval; however, changes in live weight from calving to the beginning of the breeding season and BCS at the end of the breeding season had no effect on reproductive performance.[21] Changes in postpartum nutrient intake may benefit thin (BCS <4) more than adequately nourished cows. Pregnancy rates were evaluated in cows whose calves were removed for 48 hours at initiation of breeding. Cows with BCS less than 4 at calving that were fed to lose weight after calving had decreased pregnancy rates at 40 and 60 days after breeding compared with cows fed to gain weight after calving, cows fed to maintain their weight after calving, or cows fed to lose weight but with 2 weeks of increased energy intake before breeding (flushing). The ration fed after calving did not alter the pregnancy rate in cows that calved with BCS greater than 5.[22] Conversely, cows fed to gain 0.9 kg/day after calving had higher first-estrus pregnancy rates and higher serum IGF-I, leptin, and glucose concentrations post partum than cows fed to gain 0.5 kg/day. Condition score at calving (group means 4.4 versus 5.1) did not influence hormonal status after calving.[24]

The timing of the calving season may modify the influence of nutrition on reproductive performance. BCS at calving may be more critical in spring-calving herds, whereas changes in BCS from calving to breeding may be more important in fall-calving cows owing to differences in availability of feed.[25]

All increases in nutrient supply before and after calving should be evaluated relative to cost of added feed versus potential increase in reproductive performance.

Table **58-2**

Body Condition at Calving and Return to Estrus after Calving in Beef Cows

		% OF COWS CYCLING	
BCS at Calving	No. of Cows	60 Days after Calving	90 Days after Calving
Thin (1–4)	272	46	66
Moderate (5–6)	364	61	92
Good (7–9)	50	91	100

BCS, body condition score.
Data from Whitman, Colorado State University, 1975.

Body Condition Scores

Body condition scoring is a visual and tactile appraisal of muscle and adipose tissue. Condition scores allow more precise ration assessment or formulation and predict reproductive performance. Body condition may more accurately estimate tissue reserves than body weight. Weight loss during the periparturient period is associated with loss of fetus, fetal fluids, and placenta, whereas increases in weight are associated with digestive tract fill needed to support lactation and an increase in udder size. Most beef cattle condition scoring systems use a 9-point scale, with BCS 1 being extremely thin and BCS 9 being extremely obese.[22] Body condition and weight were evaluated in 11,301 Angus cows at weaning, and a positive correlation was found between BCS and body weight. BCS accounted for only 16% of the total variation in weight, however.[26] Herd, year-month, and cow age also influenced body weight. Body weight changes among condition scores were not constant; 50- to 70-pound differences were found between condition scores lower than 5, and 85- to 100-pound differences were found between condition scores greater than 5 (Table 58-3). Descriptions of BCS 3 through 6 from several published studies are presented in Table 58-4.

Table **58-3**

Body Condition and Associated Weight in Angus Cows at Weaning

BCS	Body Weight (kg)	Change from Previous BCS (kg)
2	448	—
3	473	25
4	495	22
5	525	30
6	564	39
7	604	40
8	650	46

BCS, body condition score.
Data from Northcutt SL, Wilson DE, Willham RL: Adjusting weight for body condition score in Angus cows. *J Anim Sci* 1992;70:1342.

Protein

The effect of protein on reproductive function is less clear than is the effect of energy. An association between increased protein intake, especially rumen-degradable protein, and increased early embryonic death has been reported. Energy deficiency or protein-energy imbalance, however, may be as critical as the protein excess.[4] Supplementation with 50% rumen-undegradable or bypass protein after calving did not alter pregnancy rates in cows, compared with pregnancy rates in cows fed 25% bypass protein (88% and 86%, respectively).[27] Supplementation with 250 g of bypass protein (blood meal, corn gluten meal, and soybean meal) after calving increased the percentage of first-calf heifers that conceived during the first estrous cycle compared with heifers supplemented with 250 g of degradable protein (wheat mill run, soybean meal, and urea). Supplementation with bypass protein, however, did not affect the pregnancy rate for the breeding season.[28] Pregnant beef heifers on winter range and hay were supplemented to meet either metabolizable protein requirements or crude protein requirements during gestation. Supplementation to meet metabolizable protein improved 2-year-old pregnancy rates (91% versus 86%) over the 2-year study. The authors suggested that the response to MP supplementation on reproduction was not by improved weight or BCS change before calving and the response may involve endocrine mechanism.[29]

Fat Supplementation

Fat supplements provide an energy-dense source of calories or may provide specific fatty acids that influence reproductive function. Willams and Stanko[30] reported multiple reproductive benefits of fat supplementation of beef cows. Supplemental fat fed during the early postpartum period enhanced luteal function by reducing the incidence of short cycles and prevented a postpartum decline in growth hormone. Linoleic acid inhibits the production of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by the uterus. Higher dietary fat levels may increase the concentration of cholesterol—which is a precursor for progesterone—in the blood.[30]

Fat supplementation also may play a role in the development of prepubertal heifers. Prepubertal heifers fed safflower seeds as a supplemental fat source from 254 days of age until puberty or breeding season (control diet 1.9% fat, supplemented diet 4.4% fat) had increased serum cholesterol on days 84 and 162 and increased serum progesterone concentrations 7 to 10 days after observed estrus. Heifers in this study were sired by bulls from three breeds: Hereford, Limousin, and Piedmontese. Sire breed was a factor in percentage of heifers that were pubertal at the beginning of breeding and puberty age during the entire study. Sire breed–diet interaction was significant for percentage of heifers pubertal at the beginning of breeding. Of interest, an increased number of Piedmontese-sired heifers on high-fat diets were pubertal at the beginning of breeding (97.6% versus 76.2%), whereas a decreased number of Limousin-sired heifers on high-fat diets were pubertal at the beginning of breeding (60.5% versus 69.8%). The authors concluded that the effects of supplemental dietary fat may be breed-dependent.[31]

Role of Minerals and Vitamins

Deficiencies of almost all nutrients have been thought to cause infertility, yet few have been proved to do so in cattle.[4] Dietary deficiencies of cobalt, copper, iodine, manganese, phosphorus, and selenium, as well as excesses of molybdenum, have been reported to cause infertility by one of three mechanisms: (1) decreased activity of rumen microorganisms with depression in digestibility; (2) alteration of enzymatic action, which involves energy or protein metabolism or alteration of hormone synthesis; or (3) inability to maintain the integrity of the cells of the reproductive system.[4] In addition, trace element deficiencies decrease immunocompetence, which potentially increases the risk of infectious causes of infertility.

Deficiencies of copper, selenium, and manganese have been reported to reduce fertility through altered embryonic survival and to delay estrus or puberty.[32] Cows in one herd supplemented with trace element and vitamin boluses that provided an estimated 2 mg selenium, 2 mg cobalt, 138 mg copper, 113 mg zinc, 71 mg manganese, 2.1 mg iodine, 4644 IU vitamin A, 929 IU vitamin D, and 9 IU vitamin E per day had a calving rate of 93%, compared with 64% for unsupplemented cows. In a second herd, duration of calving season was reduced from 105 days to 49 days in supplemented cows.[33] Vitamin E supplementation for 6 months in heifers was reported to increase the pregnancy rate from 33% to 58% to 83%.[34] Trace element deficiencies often manifest as nonspecific disorders with impaired reproduction as one component. Thorough analysis of feeding programs and analysis of biologic samples for trace elements should allow the practitioner to evaluate the role of trace elements in infertility.

Table **58-4**

Clinical Features with Body Condition Scores (BCSs) in Beef Cattle

	CLINICAL FEATURES			
Study	**BCS 3**	**BCS 4**	**BCS 5**	**BCS 6**
Richards et al.[22]	*Thin:* Ribs are still individually identifiable but not quite as sharp to the touch. There is obvious palpable fat along spine and over tailhead with some tissue cover over dorsal portion of ribs.	*Borderline:* Individual ribs are no longer visually obvious. The spinous processes can be identified individually on palpation but feel rounded rather than sharp. Some fat cover over ribs, transverse processes, and hip bones.	*Moderate:* Cow has generally good overall appearance. On palpation, fat cover over ribs feels spongy, and areas on either side of the tailhead now have fat cover.	*High moderate:* Firm pressure now needs to be applied to feel spinous processes. A high degree of fat is palpable over ribs and around tailhead.
Westendorf et al.[43]	*Thin:* Ribs still identifiable but not as sharp to the touch. Some fat along the spine and over the tailhead.	*Borderline:* Individual ribs are no longer obvious. The spine still feels rounded rather than sharp. There is some fat cover over the ribs and hip bones.	*Moderate:* Good overall appearance. Fat cover over the ribs feels spongy, and areas on either side of the tailhead have fat cover.	*High moderate:* Firm pressure must be applied to feel the spine. A large amount of fat is present over the ribs and around the tailhead.
Corah[44]	Beginning of fat cover is seen over the loin, back, and foreribs. Backbone is still highly visible. Process of the spine can be identified individually by touch and may still be visible. Spaces between the processes are less pronounced.	Foreribs are not noticeable; 12th and 13th ribs are still noticeable to the eye, particularly in cattle with a large spring of rib and ribs wide apart. The transverse spinous processes can be identified only by palpation (with slight pressure) to feel rounded rather than sharp. Full but straightness of muscling in hindquarters.	12th and 13th ribs are not visible to the eye unless the animal has been shrunk. The transverse spinous processes are rounded, though this can be felt only with firm pressure; the roundness is not visually apparent. Spaces between the processes are not visible and distinguishable only with firm pressure. Areas on each side of the tailhead are fairly well filled but not rounded.	Ribs are fully covered, not noticeable to the eye. Hindquarters are plump and full. Noticeable sponginess to covering of foreribs, and on each side of the tailhead, can be felt. Firm pressure is required to feel transverse processes.
Pruitt and Momont[45]	Outlines of all ribs are visible.	Outlines of 3–5 ribs are visible.	Outlines of 1–2 ribs are visible.	No ribs are visible.
Wiltbank[46]	*Thin:* Fat along backbone and slight amount of fat cover are present over ribs.	*Borderline:* Some fat cover over ribs is present.	*Moderate:* Fat cover over ribs feels spongy.	*Moderate to good:* Spongy fat over ribs, and fat is beginning to be palpable around tailhead.

Endophyte-Infected Tall Fescue

Pregnancy rates reported for cows grazing endophyte-infected *(Acremonium coenophialum)* tall fescue are 67%, 55%, 33%, 80%, 55.4%, and 39%, whereas pregnancy rates for control cows grazing uninfected or low-endophyte fescue pastures are 86%, 96%, 93%, 90%, 94.6%, and 65%, respectively.[35] Plasma prolactin concentrations are decreased in cows that consume endophyte-infected fescue, yet LH concentrations are normal. The endophyte generates toxins such as ergovaline, which may affect the cow's ability to maintain pregnancy.[35]

NUTRITION AND MALE REPRODUCTION

The associations between bull fertility and nutrition have been reviewed.[36] Rate of gain after weaning and level of nutrition influence the weight and age at which bulls reach puberty. Young bulls maintained on low planes of nutrition had reduced testicular growth, ejaculate volume, sperm production, and seminal vesicle development. Conversely, overfeeding energy to young bulls can decrease reproductive performance as a result of increased fat deposition in the scrotum or the pampiniform plexus. Severe reduction of protein in rations fed to young bulls decreased sperm production capacity. Refeeding of nutritionally stressed growing bulls has improved reproductive performance in some trials but not in others. Mature bulls appear more resistant to dietary stressors; however, overfeeding and severe protein restriction have resulted in decreased libido.[36]

Zinc deficiency in ruminants impairs fertility in males, as evidenced by reduced testis size in zinc-deficient calves.[37] Selenium is present as glutathione peroxidase in seminal plasma. Semen from bulls with low selenium concentrations had lower motility after thawing than did semen from bulls with higher selenium concentrations.[38] Yearling beef bulls treated with sustained-release selenium boluses had increased sperm motility when compared with untreated bulls.[39]

Gossypol, a compound found in cottonseed that reduces fertility in male rats and humans, immobilizes bull spermatozoa in vitro.[40] Gossypol fed at rates of 6 and 30 mg/kg of body weight for 60 and 42 days, respectively, did not affect seminal quality, quantity, or spermatogenesis in yearling Holstein bulls when compared with control animals fed soybean meal rations.[41] Rations containing 60 mg of gossypol per kilogram of body weight (whole cottonseed), 6 mg/kg (cottonseed meal), and 0 mg/kg (soybean meal) were fed to Brahma bulls from weaning to puberty. Age at puberty was increased and 196-day weight gain was decreased in bulls fed whole cottonseed compared with bulls fed cottonseed meal. The age at puberty and 196-day weight gain were intermediate in the group fed soybean meal; therefore, gossypol did not express its effects in a dose-dependent manner. Electroejaculated semen quality and quantity at puberty did not differ among treatment groups; however, luminal diameters of the seminiferous tubules were larger, germinal epithelium was thinner, and germ cell layers were fewer in bulls fed gossypol.[42] Gossypol, therefore, alters microanatomy of the testes but seems to have minimal influence on the quantity and quality of semen from young Holstein or Brahma bulls.

SUMMARY

Research trials that investigate the relationship between nutrition and reproduction may be inadvertently biased to give false negative results because of the number of cows needed to detect small differences in pregnancy rates. An investigator needs 335 cows in each group to detect a difference between an 85% pregnancy rate and a 90% rate at $P < 0.05$. Conversely, a trial with 50 cows per treatment group can detect only a difference between a 75% pregnancy rate and a 90% pregnancy rate at $P < 0.05$. Studies with small numbers per group, therefore, should be evaluated carefully.

In simple terms, the developing fetus has a high priority for nutrients, lactation is a secondary priority that will decrease with decreased intake, and conception has a low priority and tends not to occur until other requirements such as maintenance, growth, and lactation are satisfied. In addition, suckling inhibits resumption of the estrous cycle. Nutrient supply to the cow before parturition and the resulting BCS at calving appear to be the most important factors in determining reproductive performance. Postpartum energy intake above maintenance can increase reproductive success in thin cows. Thus, the management decisions of when to place the bull with the cows and when to wean the calves are critical and should match the available nutrient supplies to the demands of the cows.

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CHAPTER 59

Heifer Development: Nutrition, Health, and Reproduction

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Replacement heifer development is a critically important area of beef production in which veterinarians can provide valuable production medicine advice to their clients. Development costs for preparing a heifer to calve at 24 months of age average about 31% of her lifetime expenses, whereas delaying first calving to 30 or 36 months increases the cost of development to 42% or 46% of her lifetime costs, even if her lifetime productivity is extended by 6 to 12 months.[1]

Not only does the group of replacement heifers need to calve at a mean of 24 months, but the distribution of calving should result in most if not all of the heifers calving early in the calving season.[1] In order to reach this goal, the heifer development program should ensure that most heifers in the replacement pool reach puberty at least 42 days before the start of breeding, because the conception rate to first service is lower on the pubertal estrus than on the third estrus.[2,3]

Many producers put additional pressure on heifers to reach puberty at a young age by breeding them 3 to 4 weeks earlier than the mature cowherd. The stress of calving is greater on heifers than on older cows and calving difficulty is more likely in this group. Thus, breeding replacement heifers essentially one heat cycle earlier than the mature cows allows the producer to concentrate on the heifers at calving. In addition, the length of time from calving to the resumption of cycling is longer in heifers than in cows.[2] Therefore, calving heifers earlier than mature cows gives the heifers the extra time they need to return to estrus and be cycling at the start of the subsequent breeding season.

A heifer development program that is designed to start breeding replacements 28 days earlier than the mature herd, and that strives to have a high percentage of heifers reaching puberty 42 days before the start of breeding, needs to have the group reaching puberty by $12\frac{1}{2}$ months of age.

The beef heifer has reached puberty when she is able to express estrous behavior and ovulate a fertile oocyte. The onset of puberty is influenced primarily by genetic factors governing age and weight at onset specific to the breed.[2,3] The age at puberty can be decreased by selecting for breeds with a younger age at puberty, selecting within a breed for younger age at puberty, or crossbreeding with another breed that has a similar or younger age at puberty. Other factors also can have some influence on the onset of puberty and include exposure to bulls, time of year, and exposure to progestogens.[2–4]

A SYSTEMATIC APPROACH TO HEIFER DEVELOPMENT

The primary objectives for successful heifer development are for the heifer to calve early, give birth to a healthy, vigorous calf, and rebreed. A comprehensive set of guidelines for replacement heifer development that coordinates established management practices known to be beneficial to appropriate heifer development can be developed using a total quality management approach.

A comprehensive health and vaccination program starting at or before weaning should be administered under the advice and guidance of the veterinarian to ensure proper use of health products according to label directions. The health program is focused on maintaining good health and providing adequate protection against the major diseases that cause reproductive losses and reduced reproductive performance in cattle.

Prebreeding examinations serve as a monitoring point to evaluate the postweaning to prebreeding phase of heifer development. These examinations are scheduled in advance of the breeding season to identify deficiencies and determine readiness of heifers for breeding. These examinations should include determination of weight assessment of body condition, assignment of reproductive tract score (RTS), pelvic measurements, and visual observations for structural soundness.

Early-pregnancy examinations should be scheduled to determine the success of the breeding program and to determine fetal age. This can be especially useful in herds in which artificial insemination (AI) is used. Allowing a minimum of 2 weeks between the AI period and natural service permits the examiner to distinguish AI-bred heifers from natural service–bred heifers.

Each client should receive individual and summary data from the pregnancy examinations. These data include stage of gestation (in days) for each heifer and a projected calving date based on the observation. Producers utilizing synchronization and AI can be provided with synchronization response and AI conception rates. The summary data also should include total pregnancy rates and pregnancy rates by 20-day intervals.

Ration Formulation

For heifers to reach puberty by 12 to 13 months of age, they must receive adequate nutritional intake to signal the body that the "luxury" of reproduction is attainable. Once puberty is attained, nutrition must be at a level that allows the heifer to continue cycling, ovulate a viable oocyte, and establish pregnancy. Nutritional demands of heifers during pregnancy exceed those of mature cows because the heifer is partitioning nutrients for her own growth, as well as for fetal growth and development. This increased demand for nutrients continues through early lactation, when the beef female has her highest nutritional requirements. Deficiency of energy or protein for extended periods during the first $2\frac{1}{2}$ years of life will have a negative impact on fetal development, calf viability, milk production, and rebreeding for the next pregnancy.

Social interaction within beef herds dictates a lower status for smaller, younger animals such as replacement heifers. If harvested forage or supplements are fed to groups that contain both mature cows and replacement heifers, the intake of heifers is negatively affected by dominance aggression displayed by mature cows. Because of this social constraint, heifers must be fed separately from the mature cows in order to obtain necessary nutrients.

Weaning to Breeding Nutrition

The target weight concept is based on the fact that *Bos taurus* breed heifers such as Angus, Hereford, Charolais, and Limousin are expected to reach puberty at approximately 60% of mature weight.[2] Dual-purpose breed heifers such as Braunvieh, Gelbvieh, and Red Poll (selected for both meat and milk production) tend to reach puberty at about 55% of mature weight. *Bos indicus* heifers, most commonly Brahma or Brahma-cross, are older and heavier at puberty than the other beef breeds, reaching puberty at about 65% of mature weight.[3] The target weight for heifers can be based on the average mature weight for the herd, or it can be determined by using the frame score to predict mature weight[4] (Table 59-1). Once the target weight is known and the number of days until the start of the breeding season (or until a mid-development ration change) is determined, the rate of gain needed is a simple calculation.

Meeting the target weight, but not grossly exceeding it, is important for heifer fertility and production. Developing heifers on a high plane of nutrition (both energy and protein) from weaning to breeding results in earlier puberty,[3] improved udder development,[3] and increased conception rates[4] compared with a low plane.

Although hitting the target weight at the start of the breeding season is important for fertility and future productivity, weight gains do not need to be consistent throughout the weaning to breeding period. Freetly and associates showed that so long as replacement heifers grow to meet a minimal body weight before mating, a period of limit feeding, followed by full feeding to capture compensatory gain, may be used to decrease the amount of feed required for heifer development without a decrease in the ability of the heifer to conceive, or a decrease in her calf's growth potential; however, first-calf survival may be affected.[3]

To ensure that the target weights and body condition scores are being met, a subgroup of the heifers should be weighed and scored for body condition at reasonable intervals (such as monthly), to confirm that targeted gains are being reached. If gains are not near target levels, the ration should be adjusted accordingly.

Breeding through Midgestation Nutrition

The target weight concept can be integrated into planning the nutritional requirements through pregnancy. A heifer should weigh 80% to 85% of her mature weight at the time of calving as a 2-year-old. Energy and protein requirements (National Research Council [NRC] estimates) for growing heifers during mid-gestation should be used to formulate rations that allow heifers to maintain body condition and progress toward target calving weight. Economic considerations may favor limited weight gain or even weight loss during mid-gestation in mature beef cows, but because of higher nutrient demands of heifers, little or no decrease in body condition should occur during the first pregnancy.

Last 60 Days of Gestation Nutrition

The nutritional demands of pregnancy increase as gestation progresses. These demands increase not only as a

TABLE **59-1**

Mature and Puberty Target Weights for Heifers of Different Frame Sizes

Frame Score	Expected Mature Weight (lb)*	55% of Mature Weight (lb)	60% of Mature Weight (lb)	65% of Mature Weight (lb)
2	953	524	572	619
3	1027	565	616	668
4	1100	605	660	715
5	1173	645	704	762
6	1247	686	748	811
7	1320	726	792	858

*Data from Fox DG, Sniffen CJ, O'Connor JD, et al: A net carbohydrate and protein system for evaluating cattle diets. III. Cattle requirements and diet adequacy. *J Anim Sci* 1992;70:3578–3596.

result of fetal growth but also because of uterine/placental growth and metabolism involved with the fetal-maternal interaction and the exchange of nutrients and waste.

In one study, heifers calving in body condition scores (BCSs) of 4, 5, or 6, respectively, had calves with progressively heavier birth weights, but dystocia score was not influenced by BCS at calving.[4] Heifers with greater weight gains ante partum had calves with heavier actual and 205-day adjusted weaning weights than did heifers with moderate weight gains.[18] Greater BCS at calving resulted in more heifers in estrus and more heifers pregnant by 40 and 60 days of the subsequent breeding season.[18] Thin females should be fed levels during the last third of pregnancy to achieve a targeted BCS of 6 or higher at calving, whereas those in moderate-high to high body condition at 90 days before calving should be fed levels to maintain body reserves.

First 80 Days of Lactation Nutrition

During the first 80 to 100 days after parturition, the heifer must continue to grow at about 0.5 lb per day, support lactation for a suckling calf, resume estrous cyclicity, and conceive for her second pregnancy. The maintenance requirement for lactating heifers averages about 20% higher than that for nonlactating heifers, with actual maintenance requirements being greatly affected by level of milk production. Marston and colleagues[3] illustrate the importance of adequate body condition at calving in that supplementation of energy or protein after calving had little effect on subsequent pregnancy rate. The period of time between calving and rebreeding is fairly short, only 82 days to maintain a 365-day calving interval, and during this time the cow has her highest nutritional demand due to lactation. Because of these factors, weight gain or body condition increase is difficult in the early postpartum cow. Lalman and co-workers[4] found that feeding high-energy diets post partum to thin heifers reduces the negative effects of prepartal nutrient restriction but does not completely reverse those effects.

Use of Progestogens

Progesterone and synthetic progestogens induce puberty in heifers, and management systems that capitalize on this result have been developed. Short and colleagues showed that more prepubertal heifers (8.5 months of age and weighing 249 kg) given a progesterone implant for 6 days plus an injection of estradiol-17β 24 hours after implant removal showed estrous behavior and ovulated within 4 days than heifers treated with estradiol-17β alone.[5] A commercially available synthetic progestogen is melengestrol acetate* (MGA). Studies have demonstrated the ability of MGA to induce puberty in heifers, especially heifers near the age and weight requirements for spontaneous induction of puberty. Conception rate at first service for heifers that attained puberty while being treated with MGA administered orally for 14 days, followed by prostaglandin $F_{2\alpha}$ given as an intramuscular injection 17 days after the final day of MGA feeding, was not different from that for control heifers that attained puberty during the same period.[5]

*The Upjohn Co., Kalamazoo, MI.

Use of Ionophores

Ionophores originally were cleared for use to improve the feed efficiency of feedlot cattle on high-concentrate diets[2] and to improve pasture cattle gains.[6] Now, ionophores also are cleared for use in replacement heifers. Inclusion of ionophores in heifer diets has been shown to increase the number of heifers that had reached puberty by the start of the breeding season, decrease the age at puberty, decrease the weight at puberty, increase the corpus luteum weight, and increase the amount of progesterone produced.[6–8] The decrease in age at puberty was independent of improved average daily gain and increased body weight.

Effect of Growth Implants

Implanting suckling calves with anabolic growth promotants is a highly profitable practice used by cow-calf operators to increase weaning weights of calves intended for slaughter. Research on the effect of implanting heifers that are later saved for replacements on percentage cycling and conception rates has been somewhat inconsistent, with results ranging from negative[6,7] to positive.[2] When nutritional levels are adequate to sustain the anabolic effects on weight gain, implants have been reported to have no negative effects.[7] Negative results were most likely to occur when implants were placed at birth, or when heifers were implanted with anabolic agents three times between birth and puberty.[28,29]

Numerous studies have shown that heifers implanted with anabolic growth promotants at 2 to 3 months of age have a larger pelvic area as yearlings than do controls without implants.[7,8,31] This increase ranged from 10 to 29 cm^2. A few studies have followed the heifers to calving at 2 years of age to determine whether the larger pelvic areas were maintained. These studies showed that much of the advantage for implanted heifers seen as yearlings was lost by the time they were ready to calve; the advantage was only 3 to 9 cm^2, compared with controls with no implants.[31,32]

Use of Anthelmintic Treatment

Internal parasites can have a negative impact on virtually all production characteristics of beef cattle, including gains from weaning through the first pregnancy.[7,8] Minimizing the negative impact of internal parasites improves the efficiency of gain for replacement heifers. Improved gain increases body weight and hence the number of heifers cycling at the beginning of the breeding season.[27,29] It is interesting to note, however, that improvements in reproductive response in replacement heifers treated with anthelmintics may not be due solely to reaching target weights faster than nontreated heifers. It is noteworthy that Larson and associates found the correlations between weight gain or prebreeding heifer weight and puberty in ivermectin-treated heifers

approached zero, indicating that the gain response does not fully explain the earlier onset of puberty.[35] Purvis and Whittier also showed that decreased age and weight at puberty in ivermectin-treated heifers compared with controls was not due to improved average daily gains.[27] Therefore, other pathways affecting onset of puberty, besides weight gain, are being stimulated due to treatment with ivermectin and possibly other anthelmintics.

Assessment of Growth and Maturity

Body Weight

Yearling weight should approach or exceed the target weight in order to have a high percentage of a group of heifers pubertal by the start of the breeding season.

Reproductive Tract Scores

The reproductive tract scoring system was developed as a practical method for determining onset of puberty in production herds. This system subjectively classifies pubertal status using size of the uterus and ovaries estimated by palpation per rectum.[4] A reproductive tract score (RTS) is assigned to each heifer using a 5-point scale on which a score of 1 is considered to indicate an immature tract and scores of 4 and 5 are considered to indicate a cycling tract (Table 59-2).

An RTS of 1 is used to describe heifers with infantile reproductive tracts that are not near the time of puberty when palpated. These heifers have small, flaccid tracts and small ovaries with no significant structures. Heifers assigned an RTS of 2 have slightly larger uterine diameter, but tone is still lacking and the ovaries have very small follicles. Heifers described as having an RTS of 3 have some uterine tone and larger uterine diameter than heifers with more immature scores. These heifers are subjectively evaluated as being close to cycling (within 6 weeks). Heifers assigned either a score of 4 or 5 are considered to be cycling, as indicated by good uterine tone and size and easily palpable ovarian structures. RTS 4 is assigned to heifers that do not have a palpable corpus luteum (CL), despite the presence of large follicles, either because they are in their pubertal cycle or because they are in a stage of the estrous cycle in which a CL is absent. Heifers with an RTS of 5 are similar to RTS 4 heifers in uterine and ovarian size, tone, and structure when palpated per rectum, except that a CL is present in RTS 5 heifers.

Pregnancy rates subsequent to AI after estrous synchronization differed between RTSs, with higher pregnancy rates found with progressively higher tract scores.[8] Because of the myriad variables affecting conception and pregnancy rate, however, simply having a high percentage of a group of replacement heifers assigned an RTS of 4 or 5 did not ensure a high AI pregnancy rate.

Heifers should be evaluated for RTS about 6 to 8 weeks before the start of the breeding season. If deficiencies are found, management changes instituted this far ahead of the breeding season can result in an increased number of heifers reaching puberty by the start of the breeding season. If the heifers are evaluated too far ahead of the breeding season (>8 weeks), the heifers are likely to be young and to have lower RTSs that do not constitute a true reflection of their potential to reach puberty before the breeding season.

Pelvic Area Measurement

The use of pelvic measurement at 1 year of age as a tool to decrease the incidence of dystocia has been described extensively since the late 1970s.[9] Veterinarians have used pelvic area measurements in yearlings because the major cause of dystocia is a disproportionately large calf relative to the heifer's pelvic area. The correlation between yearling and 2-year-old pelvic areas is 0.70; therefore, measuring the heifer's pelvic area as a yearling is beneficial for predicting pelvic size at the time of parturition.[38] Pelvic area is moderately to highly heritable (0.44 to 0.61), so after a few years of measuring replacement heifers and bulls used to produce replacements, producers can increase average pelvic size of the herd.[10,11]

Critics of using pelvic area measurements to decrease dystocia point out that pelvic area is also positively

TABLE **59-2**

Reproductive Tract Scores (RTSs)

		APPROXIMATE OVARIAN SIZE			
RTS	**Uterine Horn Size/Tone**	**Length (mm)**	**Height (mm)**	**Width (mm)**	**Ovarian Structures**
1	<20 mm diameter No tone (immature)	15	10	8	No palpable follicles
2	20–25 mm diameter No tone	18	12	10	8-mm follicles
3	25–30 mm diameter Slight tone	22	15	10	8- to 10-mm follicles
4	30 mm diameter Good tone	30	16	12	>10-mm follicles CL possible
5	>30 mm diameter Good tone	>32	20	15	>10-mm follicles CL present

CL, corpus luteum.

correlated to mature cow size and calf birth weight.[10,11] If producers place selection pressure on heifers for pelvic area by selecting for increasingly larger pelvic area, calf birth weight also will increase, so that the rate of dystocia is not likely to decrease.[10] A number of researchers have shown that selection based on pelvic area alone did not significantly reduce the incidence of dystocia in groups of heifers.[11,12]

Rather than using pelvic area measurement to select for maximum pelvic size, this tool should be used to set a minimum pelvic size as a culling criterion (such as 150 cm^2 at 1 year of age) without assigning preference for heifers that exceed the minimum.

Evaluation of Reproductive Soundness of Heifers Based on Weight, Reproductive Tract Score, and Pelvic Area

An effective way to evaluate the reproductive soundness of yearling heifers in a ranch setting is by using yearling weights, RTS, and pelvic area measurements together to describe the maturity and reproductive soundness of the heifer group. These three criteria are closely correlated in that within a group of heifers with similar genetic makeup, both higher tract scores and greater pelvic areas can be expected in heifers that have heavier yearling weights.

Because yearling weight, RTS, and pelvic area normally are well correlated, the practitioner or the producer should make note of heifers or groups of heifers for which that relationship is not strong. Heifers that have reached their target weight and have a high RTS but that have small pelvic areas may have a genetic predisposition for a small pelvis. This genetic input may have come from the male or the female component of the genetic makeup. As another example of lack of correlation of these normally well-correlated criteria, heifers that were implanted with a growth promotant near the time of birth may have very adequate yearling weights and pelvic areas but RTSs that indicate tract immaturity.

Pelvic area tends to increase more rapidly near the time of puberty than during the prepubertal period.[10] This knowledge is useful in evaluating pelvic area data in that a heifer that has an RTS of 5 and is of adequate yearling weight but has a small pelvis has a high probability of having a small pelvis at the time of calving as a 2-year-old, whereas a heifer with the same pelvic area that has an RTS of 2 and has not reached her target weight may very well have an adequate pelvis at calving if management changes are made so that she reaches puberty and becomes pregnant.

Biosecurity

Biosecurity is the attempt to keep infectious agents (e.g., bacteria, virus, fungi, parasites) away from a herd. One aspect of biosecurity is a vaccination program that improves the immunity of cattle against the infectious agents that they may contact. Not all diseases of cattle have commercial vaccines available, and no vaccine is completely effective at preventing disease in all situations. Therefore, other aspects of disease prevention and biosecurity are at least as important as a vaccination program. A vaccination program should be tailored to address specific risk factors and then rigorously applied to the herd. For most beef herds, the potential list of diseases in a vaccination program include brucellosis, infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), vibriosis (campylobacteriosis), and leptospirosis. Other diseases for which vaccines are available are *Haemophilus somnus* infection and trichomoniasis.

Because most infectious agents cannot live very long outside or off an animal, and because most do not travel great distances through the air, a method to keep other animals and people away from a herd will be almost entirely effective in accomplishing the goal of keeping infectious agents away. Keeping a closed herd is one method of maintaining biosecurity. A **closed herd** is one in which no cattle enter the farm and no cattle on the farm have contact with cattle from other farms. A herd is not closed if cattle share a fence with cattle from a different farm, if cattle are purchased (bulls, replacement heifers, replacement cows, stocker cattle), if cattle return to the herd after being at a performance evaluation (i.e., bull test station) or show, if bulls are borrowed or loaned, or if cattle are transported in a vehicle that transports other cattle. Using this definition, it is obviously difficult (and perhaps not desirable from a production standpoint) to maintain a completely closed herd. Including as many closed-herd protocols as possible within a farm's or ranch's management practices, however, will minimize exposure to infectious agents.

In **open herds**, additions (replacement females and bulls) should be purchased only from herds managed with a known and effective vaccination and disease testing and diagnosis program. Producers should avoid purchasing animals from unknown sources or that have been mixed with other cattle before sale. Also, additions to the herd should be isolated from the resident herd for at least 1 month before introduction to the herd. Isolated cattle should not share feeders, waterers, or air space (the optimal distance will depend on wind velocity and direction and is not well defined). During the isolation period, animals should be vaccinated with the same program as that used on the farm and screened to identify those replacements persistently infected with BVD virus using an immunohistochemistry (immunoperoxidase) test on a skin biopsy sample, or by polymerase chain reaction assay, virus isolation test, or enzyme-linked immunosorbent assay (ELISA) of serum or blood. The practitioner should work with the diagnostic laboratory to accurately interpret the tests. Some beef operations also may screen for Johne's and bovine leukosis virus (BLV).

Equipment and animals other than cattle can carry infectious diseases. Exposure of cattle to rodents, birds, cats, and dogs should be limited. Rodents and birds are a problem primarily when cattle are confined, and professional exterminators may be needed to devise an effective control plan. Salmonellosis, cryptosporidiosis, and other diseases can be transmitted by dogs and cats; therefore, keeping pet animals away from cattle is an important aspect of biosecurity.

Humans can carry infectious diseases; therefore, producers should limit access to the herd and ensure that

visitors wear clean boots and coveralls if they have recently visited other cattle operations. Trucks that deliver animals and feed, or that pick up animals (alive or dead), should remain away from the herd and away from normal traffic areas.

SUMMARY

A systematic approach to heifer development that encompasses nutritional development to ensure heifers will reach target weights, periodic assessment of growth and maturity by measuring body weight and reproductive tract maturity, and a herd biosecurity program that includes stringent vaccination and quarantine protocols will improve pregnancy rates and optimize development costs per pregnant heifer.

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CHAPTER 60

Surgical Correction of Abnormalities of Genital Organs of Cows

RICHARD M. HOPPER

Many urogenital problems in the cow mandate culling because of economic considerations and unfavorable prognosis; however in some situations the value of the animal dictates an attempt at treatment, with restoration of fertility the goal. Because most urogenital accidents or injuries occur in conjunction with pregnancy or parturition, the goal of surgical intervention may be simply to allow for delivery of the calf, or if parturition has occurred, allow for increased weight gain of the calf or cow for sale at a later date. Thus, although restoration of fertility may not always be achieved, a salvage procedure is often an economically viable option.

ANESTHETIC CONSIDERATIONS

Most procedures described here can be performed while the animal is standing, with good restraint and well-utilized local anesthesia; minimal sedation is required. Although it is understood that tranquilization does not provide analgesia, it is important to remember that with cattle, controlling anxiety is just as important. Alternatively, it is also important to consider the side effects of most sedatives. Xylazine, for example, increases uterine contractions and decreases uterine profusion and oxygenation.[1] Also, most cows have a tendency to lie down when sedated. Therefore, light sedation with properly administered regional anesthesia is preferred for standing procedures. Additionally, it is typically beneficial to administer nonsteroidal anti-inflammatory drugs (NSAIDs) to minimize postsurgical inflammation and discomfort.

The regional anesthetic blocks most commonly employed are the epidural for most procedures involving the vulva, vagina, or perineum and either the inverted-L

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or paravertebral block for the flank approach celiotomy. Additional regional anesthetic techniques that may be of value are discussed as well.

Proximal Paravertebral Block

Indications
Flank abdominal approaches, whether for a hysterotomy or for gastrointestinal procedures, are the major reason this block is utilized.

Procedure
This block anesthetizes T13, L1, and L2 as they exit the intervertebral foramen. The pertinent anatomic landmarks are the head of the 13th rib and the transverse processes of the lumbar vertebrae.[2] The lumbar area is surgically prepped. Then the skin at the three injection sites is desensitized with 1 to 2 ml of 2% lidocaine. The first site (T13 block) is about 2 to 5 cm lateral to the midline, caudal to the head of the 13th rib, and cranial to the transverse process of L1. The second site (L1 block) and third site (L2 block) are also 2 to 5 cm off the midline and cranial to the transverse processes of L2 and L3, respectively. Next, a 14-gauge needle is inserted (at a 90-degree angle) into the site to facilitate passage of an 18-gauge needle for the actual deposition of the lidocaine. At each site an 18-gauge needle that is at least 10 cm in length is inserted through the 14-gauge needle to a depth of about 9 to 10 cm ("walking" off the transverse process to a depth 1 cm beyond the process) injecting 10 ml of lidocaine there and another 8 to 10 ml as the needle is removed.

The main disadvantage of this procedure is that it can be difficult in fat cattle.

Inverted-L Block

Indications
Indications are the same as for the paravertebral block. However, this block can be performed more quickly and easily.

Procedure
This is simply a "line block" that follows the pattern of an upside-down L along the dorsal and cranial borders of the paralumbar fossa. Utilizing either a 20- or 18-gauge needle, 2% lidocaine is deposited every 3 to 5 cm along the aforementioned line. A total volume of 70 to 150 ml is typically utilized. The main disadvantages are the large volume of lidocaine required and inconsistent analgesia.

Pudendal Nerve Block

Indications
This block is most commonly used in preputial surgery in the bull, but it has applications for the cow as well. It can be helpful in chronic prolapse cases in which the bladder is also prolapsed. It is also indicated in any caudal urogenital procedure in which there is extreme tenesmus and an epidural does not seem adequate.[3]

Procedure
To perform this block, first prep the area of the ischiorectal fossa. Then by rectal palpation locate the lesser sciatic foramen. This is wrist deep and lateral. The lesser sciatic foramen is formed by the sacrosciatic ligament dorsally and the lesser sciatic notch ventrally. Following the intradermal injection of a small amount of lidocaine, a 14-gauge needle is placed through the skin in the ischiorectal fossa. Then a 6- to 8-inch 18-gauge needle is passed within the 14-gauge needle and with guidance from the hand within the rectum, the tip of the needle is placed in close approximation to the internal pudendal nerve. Then 20 to 75 ml of lidocaine is injected. Because these are paired nerves, swap hands and sides and repeat.

Sacral Paravertebral Nerve Block

Indication
The sacral paravertebral nerve block provides longer "semipermanent" analgesia following caudal urogenital tract procedures in which other regional blocks do not adequately control tenesmus and avoids the potential negative side effects of the "alcohol" epidural.

Procedure
Because blocking the pudendal, middle hemorrhoidal, and caudal hemorrhoidal nerves is most easily facilitated by blocking S3, S4, and S5 as they branch off the spinal cord, the important anatomic landmarks are the foramina that are located lateral to the dorsal midline. Any lateral movement increases the difficulty, so squeeze chute restraint and tranquilization are recommended. A caudal epidural may be beneficial in the flighty or hypersensitive individual. Clip and perform a surgical prep of the skin over the dorsal sacrum. This completed, the S3, S4, and S5 foramina are located. This is best accomplished by first identifying the sacral-coccygeal joint (the most cranial of the joints movable when the tail is raised and lowered); 1 to 2 cm lateral to this are the paired S5 foramina. The S4 foramina are about 3 to 4 cm cranial, but more lateral and the S3 foramina are an additional 3 to 4 cm cranial. A stab incision dorsal to each foramen will facilitate the introduction of a 5- to 7-cm, 18-gauge needle. When the osseous ring is entered inject the 2 to 3 ml of the alcohol or alcohol/lidocaine mixture. An effective mixture is 1 ml of 2% lidocaine and 2 ml of 95% ethyl alcohol. This should effectively decrease tenesmus, while maintaining tail viability.

High Epidural Block

Indication
This block can be utilized when the cow is to be in ventral recumbency, maximum control of the hind limbs is desired, and facilities allow for a period of recumbency following the surgery.

Procedure
This procedure is the same as that for a routine caudal epidural except that the dosage of 2% lidocaine is 60 to 100 ml. The primary advantage, immobility of the rear

legs, is also one of the prime disadvantages. Additionally, it is crucial that the head, shoulders, and thorax be elevated so that analgesia to respiratory centers does not occur.

SURGERY OF THE VULVA AND PERINEUM

Episiotomy

Indications

This procedure is performed most commonly during the management of a dystocia, when the vulva has not fully dilated or there is a fetal-maternal size disproportion, and typically in heifers. Although an episiotomy helps to facilitate fetal passage during an assisted delivery, it is also performed to prevent tearing. Surgical closure is more effectively performed following an incision than with tearing, and undue trauma is avoided.

Surgical Technique

Typically the calf's head is well into the vagina and is stretching the vulva when a decision is made to perform an episiotomy. Because the vulva is stretched so tightly even if epidural anesthesia has not already been employed, local anesthesia is rarely administered prior to the incision. Utilizing a scalpel, an 8- to 10-cm incision is made at the 10:00 or 2:00 position of the vulva, with the goal being to avoid the tearing of the vulva and perineum toward the rectum.

Following the delivery of the calf, the incision can be closed with a suture and pattern of your preference. Absorbable suture with a deep vertical mattress pattern is recommended. Absorbable suture eliminates the need for removal, although re-examination prior to breeding is recommended and the vertical mattress provides good apposition of both the submucosal and cutaneous layers. A surprisingly modest defect is created when the incision is left to heal by second intention, but because of the possibility that it may predispose the patient to pneumovagina, this is not recommended.

Aftercare

Complications, although rare, can usually be prevented by the administration of an antibiotic alone or with nonsteroidal anti-inflammatory drugs.

Caslick's Procedure

Indications

This procedure is performed primarily to correct pneumovagina. It is also performed sometimes following a colpotomy (see surgery of the uterus or surgery of the ovaries) to reduce the likelihood of postsurgical complications from an iatrogenic pneumovagina or eventration. Additionally it can be utilized with very mild cases of vaginal prolapse.

Anesthesia and Restraint

This procedure is performed with the cow standing, ideally restrained in a chute utilizing epidural anesthesia or a local infusion of lidocaine in the vulvar lips in the areas to be incised.

Surgical Technique

Utilizing scissors, a thin strip of tissue is removed from the dorsal one third to one half of the mucocutaneous junction of each vulvar lip. This can be facilitated by the injection of the lidocaine described previously. The lips of the vulva are then sutured together with a suture and pattern of the surgeon's choice. Complications can result from overly aggressive closure, specifically causing a urine "backsplash" effect, but the procedure usually achieves its intended purpose.

Repair of Perineal Lacerations and Rectovaginal Tears

Indications

Perineal lacerations in cattle most frequently result from excessive traction or an attempt to deliver a fetus through a nondilated birth canal. Unlike the mare, spontaneous perineal lacerations are uncommon in cows. First-degree lacerations involve only the mucosa of the vulva or vestibule and heal in many cases without treatment. Deeper lacerations may be complicated by prolapse of perivaginal fat and by bacterial infection of the genital organs or the urinary bladder. Treatment with systemic and local antibiotics is indicated in cases of infection.

Second-degree lacerations are deeper than first-degree lacerations and involve the entire wall of the vestibule and rectum and a portion of the perineal body but do not compromise the rectum or anus. The tissues are usually devitalized and contaminated by bacteria; thus, a period of 6 to 8 weeks should be allowed for healing before surgical closure of the defect. If second-degree lacerations are not corrected, incompetence of the vestibular sphincter allows aspiration of air and feces into the vaginal canal, which commonly leads to infertility.

Third-degree lacerations involve the vagina and the rectum as well as the perineal body and the anal sphincter and leave the patient with a common opening for the digestive and reproductive tracts. This is also termed as a rectovaginal tear. Considerable tissue damage and bacterial contamination accompany this injury, and most surgeons recommend that 6 to 8 weeks be allowed before attempting to correct the damage. Fecal contamination of the vagina, the cervix, and the uterus frequently, but not always, results in infertility until the defect is repaired.

Anesthesia and Restraint

Perineal lacerations can be repaired with the patient restrained in a stanchion or squeeze chute under epidural anesthesia in most cases. Light sedation may be needed in some cases to minimize movement by the patient.

Surgical Technique

Second-degree lacerations can be repaired by débriding the margins of the wound and bringing the dorsal portion of the vulvar labia into apposition with nonabsorbable suture material.

Several operative techniques have been described for surgical repair of third-degree perineal lacerations. The objective common to all procedures is to rebuild the shelf

of tissue between the rectum and the vestibule and restore the integrity of the perineal body.

In contrast to mares, the feces of cows are soft, and most surgeons do not withhold feed and water prior to surgery. After the rectum is manually emptied of feces, the tail is tied away from the surgical field and the area prepared for aseptic surgery. A tampon made of a 4-inch stockinette filled with cotton can be placed deep in the rectum and tied to the tail with umbilical tape to assist in control of fecal soiling of the surgical site. Exposure of the surgical area can be achieved by placing retraction sutures on both sides of the anal sphincter and in both vulvar lips. Tension can be exerted on the sutures by assistants, or the sutures can be anchored to the skin.

A technique originally developed in mares has been modified and successfully used to repair third-degree perineal lacerations in cows. A horizontal incision is initiated along the junction between the rectal and vestibular mucosa from the dorsal commissure of the vulva to the shelf that lies between the intact rectum and the vestibule. The rectal and vestibular tissues are separated and the incision continued caudally at the same level to the dorsal vulvar commissure on the opposite side. Flaps of tissue, which will be used to separate the rectum and vestibule, are then formed by dissecting the vestibular mucosa on both sides ventrally for a distance of 3 cm. At the cranial limit of the laceration, the rectum and vestibule are separated for a distance of 4 to 6 cm.

Closure of the laceration is begun by the placement of two or three Lembert sutures of number 3 absorbable suture material transversely in the vestibular submucosa in the area of separation between the vestibule and rectum. Modified Lembert sutures are then placed at 1-cm intervals to appose and invert the vestibular flaps. The needle is first introduced into the perivestibular tissue, then into the vestibular submucosa in the dissected flap. The suture is then carried across the laceration and reintroduced into the vestibular submucosa of the opposite flap and finally continued into the perivestibular tissue on the opposite side and tied. The suture line is continued until the defect is closed to the level of the dorsal commissure of the vulva. The rectal mucosa is avoided in placement of the modified Lembert sutures. Concurrently, a continuous horizontal mattress suture is used to appose and seal the vestibular mucosa. Two or three modified Lembert sutures are placed, followed by two or three bites of the horizontal mattress suture until the shelf between the rectum and vestibule is re-established. The perineal body and dorsal portion of the vulvar labia are then débrided and apposed with interrupted sutures to restore their integrity and prevent aspiration of air and contaminants into the reproductive tract.

Aftercare

Procaine penicillin G (22,000 IU/kg, IM or SC, every 12 hours) is administered for 4 to 5 days after surgery. NSAIDs can be used for a short time after surgery to minimize dyschezia. A gentle digital examination of the vaginal side of the surgical site can be performed to assess healing after 2 weeks. Palpation per rectum should be avoided for at least 30 days. Uterine infections secondary to fecal contamination should be treated as indicated. Natural mating should not be permitted for at least 6 weeks after surgery or, because manipulation of the rectum is required, artificial insemination for at least 8 weeks.

The prognosis for successful repair and restoration of reproductive function is good, but rectovestibular fistulae may form in some cases. Less commonly, wound dehiscence results in partial or complete failure of the closure. Small rectovestibular fistulae may not result in infertility in all cases. Perineal lacerations do not commonly recur at the subsequent calving.

SURGERY OF THE VAGINA AND CERVIX

Urethral Extension

Indications

Urovagina or urine pooling is encountered less commonly in cows than in mares and is frequently a sequela to obstetric trauma. The cervix falls below the pelvic floor, which allows urine to flow forward and pool in the cranial vagina rather than flow caudally through the vulvar cleft. Infertility due to the spermicidal action of urine and chronic endometritis may follow. A procedure for creating an extension of the urethra in mares has been modified and adapted for use in cows.

Anesthesia and Restraint

Extension of the urethra is performed in standing animals under epidural anesthesia.

Surgical Technique

After preparation of the perineum and vagina for aseptic surgery, a Foley catheter is passed through the urethra into the urinary bladder and the bulb inflated. The vulvar labia are retracted and separated by tension on a stay suture on each side. The vaginal mucosa is then incised in an elongated U, with the apex 1 cm cranial to the urethral orifice and continued caudally along the floor of the vagina to terminate approximately 2 cm cranial to the vulvar labia. The edges of the incision are then undermined to create two flaps, which can be apposed without tension. The ventral flaps are apposed over the Foley catheter to create a shelf using 2-0 absorbable material in a continuous Lembert pattern. Some surgeons appose the submucosa with a simple continuous suture pattern of the same material, while others omit this layer. The dorsal flaps are apposed and everted into the vaginal lumen with a continuous horizontal mattress suture. The tube created to extend the urethra should be of sufficient size to allow unobstructed flow of urine. The catheter can be left in place for several days postoperatively if the patient experiences difficulty urinating, but this increases the likelihood of an ascending urinary tract infection. Fistulae are most likely to form at the cranial aspect of the newly created tube and result in continued backflow of urine; therefore, care must be taken to ensure adequate closure.

Aftercare

The patient should be observed frequently for ability to urinate normally for several days following surgery.

Endometritis secondary to urine pooling should be treated appropriately.

Replacement and Retention of the Vaginal/Cervical Prolapse

Numerous techniques have been described for both temporary and permanent repair of vaginal/cervical prolapse. This illustrates the fact that there is no "perfect procedure." Instead we have several techniques that have advantages over the others for the specific prolapse presented. Most practitioners select a technique that they feel most comfortable with and employ it the vast majority of the time but should become proficient with at least one other technique that can be employed when dictated by the case presentation.

Indications by Case Presentation

Because this is a heritable condition, repair is considered with the goal being to maximize the salvage opportunity for the client; a single exception will be discussed later. Because economics plays a role, the easiest and least costly repair is usually employed. Other considerations are severity, pregnancy status, management ability of the client, chronicity of the condition, and tissue damage from previous attempts at repair. The Bühner technique is the most commonly used procedure because it can be easily and quickly performed and because of its retention strength. A disadvantage is that when used for the cow that is pregnant the suture must be removed prior to calving.[4] Because the Bühner technique is basically a purse-string type suture, it can impair circulation and induce edema, which in turn may result in cellulitis and infection. This is often problematic in cows with *Bos indicus* genetics because they tend to have larger, more pendulous vulvas. Vulvar "prolapse pins" can be used to avoid this inflammation, but as with the Bühner method, if the cow is pregnant they must be removed prior to calving. The Minchev procedure or a modification that employs the "Johnson Button" is indicated when the cow is pregnant and management does not allow for close observation or in the case in which a Bühner suture has or might cause cellulitis and infection of the vulva. Finally in the case in which permanent retention is desired, the Winkler technique, in which the cervix is sutured to the prepubic tendon, can be employed.

Anesthesia and Restraint

Regardless of which procedure is utilized for retention of the vagina (+/– cervix and bladder) proper restraint is important. Sedation may be indicated for the extremely fractious cow. Epidural anesthesia is always indicated and a pudendal block may be helpful when the bladder is also prolapsed. Following the epidural, confirm the suspected pregnancy status of the cow by rectal palpation. Then tie the tail out of the way and clean the perineal area and the prolapsed tissues with a surgical scrub. If the bladder has been prolapsed, it is better to evacuate it by catheterization or draining via a 14-gauge (or smaller) needle. Following replacement of the prolapse it may be necessary to "re-prep" the area prior to performing your chosen retention procedure.

Bühner Technique

Surgical Technique

Stab incisions are made 1 to 3 cm dorsal and ventral to the vulva to facilitate entry and passage of a specially designed needle (Bühner needle). The needle is introduced into the ventral incision and forced dorsolaterally to the vulva and at a depth that will provide strength, until it comes out the dorsal incision. At that time the needle is "threaded" with umbilical tape and the needle is drawn back ventrally to pull the tape through. The procedure is repeated on the other side of the vulva. The suture tape is then tied at the area of the ventral incision. Although most texts describe the use of either a square or surgeon's knot, an alternative is to place a slip or bow. This allows the owner to "untie" the suture tape, check the cow to see if she is in labor and then retighten and retie it if the examination was precipitated by a "false alarm."

Prolapse Pins

Surgical Technique

These are small pins (about the diameter of a 20 penny nail and 10 cm long) with wooden pegs on the ends. These are placed transversely through and across the vulva. Typically 3, but sometimes 2 pins will provide adequate retention. Advantages are that it is external, does not cause the swelling that a Bühner will, and provides good retention. The disadvantages are that the pins must be removed prior to calving and that one must keep a supply of the pins as well as a specialized needle that facilitates passage of the pins on hand.

Minchev Procedure

Surgical Technique

This procedure provides for retention of the vagina and cervix by fixing the cranial vagina with a suture out to the external gluteal region. A modification utilizes a prolapse pin that is marketed by Jorgensen Laboratories under the name Jorvet Prolapse Kit and by Kane Enterprises as the Pro-Fix Button. Either technique can be used in the pregnant cow and allow calving without removal. After replacing the vagina or cervix, introduce the prolapse needle (this is a sharpened metal pin housed inside a rigid plastic fixture) vaginally to a point in the cranial vagina close to the cervix. This is important because pins or sutures placed midway in the vagina will allow a "partial" prolapse of the vagina, and with the straining that is stimulated by that, tearing and complete prolapse can follow. With an appreciation for the location of the rectum as well as the iliac artery and vein, place the needle through the vaginal wall and direct it out through the sacrosciatic ligament toward the skin over the gluteal area. These products have "buttons" that are fixed to the outer area and facilitate retention. The old Minchev procedure simply utilizes umbilical tape rather than a rigid pin. The tape is passed using a long needle and then fastened to the outside with a gauze stint. It is fixed on the inside vaginal wall with a button (a top from a syringe case can be used). Again, the

advantage to this procedure is that because it does not restrict the vagina, calving can proceed. Also, because adhesions often result, it sometimes provides a "permanent" repair.

Winkler Cervicopexy

Indication

Because the goal of this procedure is to provide permanent retention and the condition it corrects is known to be hereditable, case selection is important so that one avoids an actual or perceived ethical violation. The case presentation that can be determined to not be hereditable is the cow that has had multiple and continued superovulation treatments for embryo transfer. A history that reveals that none of the cows from the dam line or her heifer offspring have prolapsed offers reasonable proof that the condition was iatrogenic.

Surgical Technique

The Winkler cervicopexy in which the cervix is sutured down to the prepubic tendon provides permanent retention. Using a nonabsorbable (8 mm Braunamid) with a long S-shaped needle (13 cm long) that has been bent into a U-shape, take a transverse bite through the cervix. This can be most easily done with the cervix retracted (prolapsed cervix prior to replacement). Then after replacing the cervix pass the needle through the vagina ventrally 4 to 5 cm off the midline (placement of a urinary catheter is recommended to prevent its entrapment). Engaging the prepubic tendon, return the needle through the vagina and tie the suture ends. A modification of this procedure has been described that utilizes a second surgeon who from a lateral flank approach assists by placing and passing the needle through the prepubic tendon and back to the primary surgeon through the vagina. In the author's opinion the main difficulty in performing this procedure without assistance is not in engaging the prepubic tendon, but in the reintroduction through the vagina. If one attempts this without assistance and has trouble passing the needle back into the vagina, a colpotomy approach can be used. This will also help to better assure proper suture placement with respect to the urethra and bladder. This incision must be closed, however, as evisceration would be a possible (likely) complication due to the fact that most prolapsed cows continue to strain following prolapse repair.

Cervical Injury Repair

Indications

Cervical lacerations are frequently the result of excessive traction applied to relieve dystocia. Infertility due to incomplete closure of the cervix and subsequent bacterial contamination of the uterine cavity may follow. Lacerations that involve no more than one third of the circumference of the cervix are amenable to surgical correction.

Anesthesia and Restraint

Cervical lacerations can be repaired in standing patients under epidural anesthesia.

Operative Procedure

Surgery is delayed for 6 to 8 weeks after the inciting injury to allow swelling and inflammation to subside. The perineal area is prepared for aseptic surgery and a tampon is placed in the rectum to control fecal contamination during surgery. The cervix is retracted caudally by use of a cervical forceps or traction sutures. The edges of the defect are débrided by careful dissection and granulation tissue removed. After devitalized tissue has been removed, the cervical defect is closed in three layers. The cervical mucosa is everted into the cervical lumen using a continuous horizontal mattress pattern. The muscularis is apposed with a simple continuous pattern and the vaginal mucosa is everted into the vaginal lumen with a second layer of continuous horizontal mattress sutures.

Aftercare

Infusion of a lanolin-based antibacterial ointment into the cervical lumen for several days may reduce the formation of postoperative adhesions.

SURGERY OF THE UTERUS

Cesarean Section

Indications

Cesarean section is typically utilized when there is a dystocia caused by a fetopelvic disproportion or extreme malposture and the calf is alive. Some veterinarians choose to perform a cesarean section rather than perform a complete fetotomy, so dead calves and fetal monsters can be added as optional indications. Additionally uterine torsion, malformation of the maternal pelvis, incomplete cervical dilatation, stricture of the birth canal, and uterine rupture can often be best managed by cesarean delivery. Also if the cow has a history of dystocia or the calf is valuable, an appointment cesarean section can be scheduled a few days before term.

Anesthesia and Restraint

Cesarean section can be performed with the dam standing or in dorsal or lateral recumbency; the choice depends on the demeanor of the patient, the condition of the fetus, the facilities available, and the surgeon's preference. In general and in most cases a left flank approach with the patient restrained in a squeeze chute is the best choice.

Caudal epidural anesthesia is often used in conjunction with other forms of anesthesia and sedation for standing flank approaches. Alternatively, a high epidural can be utilized when the cow is to be placed in dorsal recumbency for a ventral midline approach. Remember that following the high epidural the cow will be ataxic following the surgery for a period of time (see earlier discussion under Anesthetic Considerations).

Paravertebral, inverted-L, and line block anesthesia are all useful to desensitize the paralumbar fossa for the flank approach in the standing patient (see Anesthetic Considerations).

Choices for sedation include acepromazine (0.044 mg/kg), xylazine 0.02 mg/kg), butorphanol (0.05 mg/kg), and combinations of these drugs. Low doses that provide

relief from anxiety, rather than deep sedation, which may encourage recumbency, are obviously preferred.

Surgical Technique

Many surgical approaches have been described for performing cesarean section in cattle. The two most commonly employed are the left flank approach, which can be performed with the patient standing or in right lateral recumbency, and the ventral midline approach.

Left flank approach. After the skin surrounding the incision site has been prepared for aseptic surgery, an incision is begun 10 to 15 cm ventral to the transverse processes of the lumbar vertebrae midway between the last rib and the tuber coxae and extended sufficiently to allow extraction of the fetus. After incising the abdominal muscles, the peritoneum is identified and incised. Opening of the abdominal cavity is usually accompanied by the sound of air entering the potential space. The rumen is displaced cranially to allow access to the other abdominal organs and can be used to advantage to block escape of the intestines through the incision. A left oblique celiotomy approach for cesarean section in standing cows has been described. The incision in the abdominal wall is begun 8 to 10 cm cranial and 8 to 10 cm ventral to the cranial aspect of the tuber coxae and is extended cranioventrally at a 45-degree angle to end 3 cm caudal to the last rib. This surgical approach permits easier access to the gravid uterus than the more traditional vertical incision in the paralumbar fossa.

One of the fetal limbs (hind limb in the case of cranial presentation of the fetus) is identified and used as a handle to deliver the uterus to the abdominal incision. It is best to exteriorize the part of the uterus that encases the distal limb, "hooking" the hock outside the incision. This will allow an incision extending from the hock down to and over the foot, which is generally sufficient to deliver the calf without tearing. When the incision is not long enough, delivery of the calf results in uncontrolled tears that complicate closure. Exteriorization of a portion of the uterus may be difficult in cases of large fetuses or uterine torsion and the practitioner may be forced to make the uterine incision intra-abdominally. Contamination of the abdomen with uterine fluids is virtually impossible to prevent if the uterus is not completely exteriorized and may occur even if it is. This is usually of little consequence when the calf is fresh (alive or recent death), but if a calf is decomposing, a potentially fatal peritonitis may result. Additionally, intra-abdominal exposure to the commonly used obstetric lubricant, J-Lube has been shown to be fatal in cattle, horses, and mice.[5] Therefore, it is recommended that because contamination of the abdomen with uterine contents is so hard to avoid during cesarean sections, that J-Lube should not be used during obstetric procedures that might ultimately result in a cesarean section. Conversely, carboxymethylcellulose, another commonly used obstetric lubricant, has been shown to be safe and to actually decrease adhesion formation.[6]

After the uterus has been incised, the fetal limbs are identified and obstetric chains are attached to facilitate delivery. The uterus is then closed with absorbable suture material. Recommended patterns are the Cushing or a modified Cushing pattern known as the Utrecht uterine suture. A single layer is sufficient if the uterine wall is healthy. Two layers can be placed if the uterine wall is friable or if leakage of uterine contents is a potential problem. Care is taken to avoid inclusion of the placenta in the suture line. Should the placenta complicate closure of the wound, it can be trimmed away from the incised edges. Do not remove the placenta unless it detaches easily.

After the uterine incision has been closed, the uterus and abdominal cavity are lavaged with large volumes of saline (with or without heparin and antibiotics) to remove or dilute clotted blood and debris. Irrigation of the uterus and the abdominal cavity with 3% glycerol in saline or a 1% solution of carboxymethylcellulose in water has been used to reduce abdominal adhesions following surgery. Administration of oxytocin into the wall of the uterus (20 units) or intravenously (100 units) after the uterine incision has been closed will hasten uterine involution and expulsion of the placenta.

Routine closure of the peritoneum and muscle layers in a continuous pattern with an absorbable suture and apposition of the skin with a nonabsorbable suture material in a pattern of the surgeon's choice completes the procedure.

Ventral midline approach. Patients can be positioned in dorsal recumbency with the head and limbs secured to maintain the patient in position. After suitable anesthesia has been applied, the skin is prepared for aseptic surgery. The incision is initiated at the level of the umbilicus and extended caudally to the cranial border of the mammary gland. An approach through the ventral body wall has the advantage of providing excellent exposure of the uterus. Exteriorization of the uterus is facilitated, and contamination of the abdominal cavity with septic uterine contents is reduced. Surgeons may find it easier to extract the fetus from dams in dorsal recumbency. Respiratory function of cattle is compromised while in dorsal recumbency; thus, surgery should be completed with dispatch. An additional disadvantage of positioning the patient in dorsal recumbency is that the surgeon is forced to perform the procedure while in an uncomfortable position.

Incisions in the ventral body wall are more likely than those in the paralumbar fossa to be complicated by postoperative hernias. Strict attention to the fundamental principles of surgery is indicated to prevent or reduce the incidence of postoperative eventration.

Hysterotomy via Colpotomy

Indication

The primary indication for this procedure is the removal of a mummified fetus from a cow that was unresponsive to medical therapy. A mummified fetus is rarely encountered and often the cow is culled. First, differentiate this from the macerated fetus, which has a poorer prognosis. If treatment is elected, first try prostaglandins. If this is not effective, try priming the cervix with estrogen followed in a few days with serial prostaglandin injections. In cases in which there is no response to these treatments a surgical remedy may be effective. Although it is possi-

ble that some of these could be removed via a hysterotomy utilizing a flank or midline approach, most "mummies" are so small that it is hard to get good exposure. A hysterotomy via a colpotomy approach can be performed[7] easier and in less time.

Anesthesia and Restraint

The cow is restrained in a chute. If possible, fast the cow, removing feed for 24 hours and water overnight. Sedation or tranquilization is dependent on the patient's personality. Administer an epidural and evacuate the rectum as effectively as possible. Then place a length of 3-inch stockinette packed with cotton within the rectum.

Surgical Technique

Clean the vulva and prepare the vagina by lavaging with a dilute disinfectant. Using dampened cotton pledgets dry or at least remove water from the vaginal vault. Creating a pneumovagina by introducing or allowing air in will facilitate the procedure. With a blade carefully guarded between finger and thumb, introduce the hand into the vagina and at a location in the anterior vagina that is dorsolateral (at 10 o'clock) to the cervix make a small stab incision. Then remove the hand and re-enter the vagina without the blade. Enlarge the incision bluntly first with the fingers and then hand until the hand can be introduced into the abdomen. At this time the surgeon can easily palpate the uterus and when the horn with the mummy is located can grasp and retract it out through the rent created in the vagina. Once it is exposed make an incision into the uterus, extract the mummy, and suture the uterus with a continuous suture pattern as for a cesarean section hysterotomy. Replace the uterus. Although suturing the vagina is considered optional and the author does not attempt to close colpotomy incisions on mares, the author does close it in cows. This can be done with a continuous pattern (the suture technique described later for uterine tears can be utilized). Fertility should be the same as in those that respond to prostaglandins.

Management of the Uterine Prolapse

Treatment of this condition begins with phone consultation when the client calls. Instruct the client to restrain the cow, as movement risks injury. If the cow is down, instruct the client to tie the cow in a way as to keep it from getting up. Then the client can either soak the prolapsed uterus in cold, salty water or wrap the prolapse with a towel that has been soaked in cold, salty water. Covering the prolapse with sugar has been recommended for its osmotic effect on the edematous uterus, but the grainy sugar crystals have the negative effect of irritating the endometrium as the uterus is being manipulated to replace it. However, it can obviously be utilized and then rinsed off after the desired effect has occurred.

Procedure

If the cow is down, place her in dorsal recumbency with her hind limbs pulled back so that she is resting, tilted forward, stifle down, and hocks up. Regardless of whether or not she is down or standing, a towel or preferably a coated mesh tray (small animal cage floor rack) can be placed under the prolapse by assistants and then raised to help you as you attempt to replace the uterus. Rinse the prolapse with a cold, hypertonic saline mixture with dilute povidone to remove dirt, hay, and other debris. This should not be excessive and in fact it is probably better to not remove placenta that is attached. Begin efforts at replacement by gently pushing the tissue closest to the vulva back in while supporting the body of the uterus at a level dorsal to the vulva. The uterus could be placed inside a garbage bag if attempts at replacement seem like they may take more than 8 to 10 minutes, as this will decrease damage to the caruncles and endometrial lining. When the uterus is replaced, fill the uterus with as much warm saline (dilute povidone) as possible to facilitate a complete straightening out of the uterine horns. Then siphon off the fluid to decrease the chance of uterine rupture. Although wine bottles, Frick speculums, and batons have all been used to accomplish this, the lavage is safer. This along with getting the cow standing is crucial, as any invagination of the tips of the uterus can result in re-prolapse or an ischemic necrosis of the uterine tip. Oxytocin (20–40 IU IM) can be administered if there is complete confidence that the uterus is in normal anatomic position. The placement of retention sutures or other retention devices is controversial as they are not necessary if replacement is complete and uterine contraction is occurring and can cause a serious complication when there is uterine invagination and attempted re-prolapse. If retention devices are in place, the uterus will not completely prolapse, but will fill the vagina within the pelvis, resulting in uterine necrosis. If retention devices are placed, and they may be indicated in the recumbent cow with an atonic uterus, then the cow must be monitored closely. Uterine boluses should not be used and uterine lavage is preferable to antibiotic infusion in the contaminated uterus. Also remember that many of these cows are also hypocalcemic, so oral or intravenous calcium may be indicated.

Uterine Tearing or Rupture

Most injures to the uterus are iatrogenic and occur during efforts to relieve dystocia. With few exceptions they occur on the dorsal aspect of the uterus (cranial to the cervix) when the obstetrician is attempting to repel a breech presentation or during the forced extraction of a large fetus. In the case of the first situation repair is secondary to removal of the calf, and so a flank cesarean section is performed, and after the calf is delivered, the uterine defect may be approached and repaired through the flank incision. In the second scenario there are several treatment options.

The etiology for each tear is different. In the case of the tear caused by "excessive" force during mutation of the calf, the uterine wall was probably already weakened by some level of pressure (ischemic) necrosis. In the second situation, a large calf, a "dry" birth canal, and forced extraction combine to create an overfolding and subsequent shearing of the uterine wall. These tears are usually 5 to 30 cm in length, although some seem almost circumferential.

Regardless of the etiology of the tear, repair may not be necessary if it is small and dorsal. Because of rapid uterine involution tears smaller than the width of a hand will usually not need repair. Treatment for these can be limited to repeated oxytocin injections for the first 24 to 48 hours and 7 to 10 days of antibiotics. If the tear is larger and must be closed, the uterus can be prolapsed to facilitate closure or repaired with a "blind" suture technique. To prolapse a uterus, administer 10 ml of epinephrine in 250 ml of saline while tugging on a uterine caruncle. It is important to monitor the heart while this is done. After the uterus is prolapsed the tear can be closed with an absorbable suture of the surgeon's preference and the uterus is replaced. An alternative is a one-handed blind suture closure.[8] Thread an atraumatic needle (size and shape is determined by preference) with a 150-cm length of No. 2 or 3 catgut. With the needle at the halfway point of the suture (doubled) make a knot at the end (knot 1) and about 25 cm from the end (knot 2). Next introduce the needle into the vagina guarding the point with the fingers until reaching the tear. At one end of the tear begin closure. After going through the uterine wall (about 1 cm lateral to the tear) run the needle between the doubled suture and cinch at knot 2. (The only purpose of knot 1 is to keep the two loose ends together.) Then close the tear with a continuous pattern until the last bite is made 1 cm lateral to the end. It is important to pull the suture tight with each throw. Then reverse and continue the closure back to the original site and tie to the tail. Uterine involution serves to cover small appositional problems. Recommended treatment following either closure includes antibiotics and oxytocin.

SURGERY OF THE OVARIES

Ovariectomy

Indications

This procedure is performed on heifers destined for grazing or feeding to suppress estrus, prevent pregnancy, and induce abortion. It has also been utilized in brucellosis control programs. A unilateral ovariectomy may be indicated to remove an ovary affected by neoplasia or surrounded by adhesions. Also removal of the ovaries prior to the death of a valuable animal and shipment to a facility that can harvest ova for in vitro maturation and fertilization is an increasing possibility.[9]

Anesthesia and Restraint

An ovariectomy is usually performed with the animal standing in stocks or a squeeze chute after feed has been withheld for 24 hours. If the ovaries are to be removed via colpotomy, an epidural is useful; if an incision in the paralumbar fossa is to be used, an inverted-L or paravertebral anesthesia can be used (see Anesthetic Considerations).

Colpotomy

Two techniques for removal of the ovaries using an incision in the cranial vaginal wall have been utilized. In patients of sufficient size, the operator's hand and arm encased in a lubricated sterile sleeve are introduced into the vagina. A scalpel handle is held in the palm, the blade guarded by the index and middle fingers and used to make a stab incision through the vaginal wall near the cervix at the 10 or 2 o'clock position. The scalpel is removed and the operator's fingers are used to bluntly enlarge the incision sufficiently to permit the hand to enter the abdominal cavity. Care should be taken to make the incision completely through the vaginal wall; if the peritoneum is not incised, it will stretch away from the operator's hand and make entry into the abdominal cavity difficult. An instrument designed to cut cloth has been found to be a useful and safe alternative to a scalpel. The blade can be guarded until the incision site has been reached and then the guard retracted. The ovaries are located in succession and withdrawn into the vaginal canal where the ovarian attachments can be severed with a spaying shears or with an écraseur and the ovary removed. The blood supply to an ovary containing a corpus luteum is substantial and hemostasis is essential. The vaginal incision is usually left to heal by second intention.

Specialized instruments have been designed for spaying heifers that are used to enter the abdominal cavity through a puncture in the vaginal wall and are manipulated by the operator's hand and arm inserted in the patient's rectum. The K-R spay instrument is a tubular device with a trocar point used to puncture the vaginal wall and enter the abdominal cavity. The ovaries are located by manipulation per rectum and placed one at a time into the instrument's cutting chamber and severed from their attachment by rotating the inner tube. Care must be taken to avoid accidental incision of the intestine. After both ovaries have been excised, the instrument is withdrawn and the ovaries removed from the chamber and discarded.

The Willis spay instrument is a stainless steel rod 48 cm long. One end is bent to form a handle while the other is flattened and has a teardrop-shaped eye with a sharpened apex used to sever the ovarian attachment. The instrument is introduced into the vagina and the vaginal wall punctured. By rectal manipulation, an ovary is placed through the eye of the instrument and the attachment severed by withdrawing the instrument with steady pressure. After the ovarian pedicle has been cut, the ovaries are allowed to drop into the abdominal cavity.

Both the K-R and Willis spay instruments are most applicable to use in prepubertal animals because enlargement of the ovary caused by the presence of a corpus luteum makes the procedure more difficult. Death following use of spay instruments has been attributed to hemorrhage and to peritonitis resulting from intestinal damage.

Celiotomy

Unilateral or bilateral ovariectomy can be performed using an incision in the paralumbar fossa. Although access can be gained from either side, the left flank is usually chosen, as the rumen reduces the possibility of prolapse of the small intestine through the incision. An

incision through the abdominal wall is centered in the paralumbar fossa halfway between the last rib and the tuber coxae. The operator's hand and arm are introduced into the abdominal cavity and the ovary is located by palpation. If a bilateral ovariectomy is to be performed, the far ovary is usually removed first and the near ovary last. Hemostasis is crucial, especially if an ovarian neoplasm or ovary containing a corpus luteum is to be removed and can be accomplished by crushing the ovarian pedicle with an écraseur or by applying an umbilical cord clamp. Ligatures can be used but are difficult to apply securely because the length of the mesovarium usually does not permit the ovary to be exteriorized through an incision in the paralumbar fossa. After the ovarian pedicle has been ligated or clamped, the ovary is excised with surgical scissors. The abdominal incision is closed using the suture material and pattern of the surgeon's choice.

Aftercare

The degree of aftercare following ovariectomy varies with the type of patient. In the case of feedlot heifers, recommended aftercare ranges from observation only to administration of antibiotics parenterally or in feed for several days following surgery. Treatment for 5 to 7 days with an appropriate antibiotic usually is recommended following therapeutic unilateral ovariectomy in adult animals. Postoperative analgesics may be indicated in selected cases.

Sequelae to ovariectomy include excessive hemorrhage and peritonitis. In cases of unilateral ovariectomy, adhesions between the remaining reproductive organs and the surrounding tissue may interfere with future fertility. Methods that minimize hemorrhage and fibrous tissue formation should be chosen if the patient is to be used for breeding.

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CHAPTER 61

Reproductive Health Programs for Dairy Herds: Analysis of Records for Assessment of Reproductive Performance

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RECORDS AND MONITORING

The effective use of records is a cornerstone of modern dairy production medicine. Records provide access to the performance results of a dairy's management and serve as a major source of diagnostic information when problems arise. As veterinary service to dairy farms has matured, practitioners have become more involved in herd-level analysis, management consulting, and problem solving. Monitoring is an essential component of any system that must respond to external influences (Fig. 61-1).[1–3] A parameter of the system is measured and compared with standards, goals, or past performance. If the parameter does not meet the goal, then plans are made and actions are taken (usually including collection of more diagnostic information). Because of both the action taken and the external influences on the system, a result is achieved. The result becomes the new status and the cycle begins again. Even though this activity is routine in most veterinary reproductive programs, in many cases it is not as fully developed or deliberately documented as might be most useful to the client.

Although the general scheme described is consistent across all types of monitoring, it is useful to distinguish between the following four general classes of monitoring.

1. **Surveillance.** Surveillance is a monitoring system designed to generate action at the first detection of a parameter. *Brucella* testing for reproductive disease control and public health is an example. The monitoring system (milk ring test or herd serology) is set such that a predetermined course of action will be taken if any indication of brucellosis is detected. These sort of monitoring systems typically play a minor role in day-to-day dairy farm management.
2. **Status monitoring.** This form of monitoring involves the measurement of a parameter and the comparison of its absolute value to a goal figure. Services per conception might be set at 3.0, with the intent that performance worse than that number will initiate further diagnostic effort or management changes. In outbreak investigation, this is the typical starting point for the veterinarian. Status monitoring simply asks, "Are things as they should be?"

   Although attempts have been made to arrive at a single "index" that will assess the performance of a dairy's reproductive program, none are adequate alone.[2] Evaluating reproduction involves a complex set of issues that are to some degree unique to the particular dairy. Attempts have been made to develop expert computerized systems to evaluate reproduction,[4] but none are in widespread use at the current time.
3. **Trend monitoring.** Typically more robust than status monitoring, tracking the trend in a parameter over time provides an added dimension of understanding to the analysis. Services per conception of 3.0 may be a problem in a herd that had been at 2.5, whereas it may be cause for celebration in a hot climate herd whose historical average has been nearer to 3.5. Trends allow for graphic display of the data and improve both client understanding and motivation.
4. **Exception monitoring.** Here, the trend of the herd as a whole is not the emphasis; instead attention is focused on displaying the individuals whose performance is substandard. In a sense, this is status monitoring within the herd, applied to individuals. Generally, this sort of detailed monitoring can be accomplished conveniently only with computerized record systems. Exception monitoring results are generally in the form of counts, graphic distributions, or action lists.

Viewed from another perspective, monitoring can be applied to two classes of information:

1. **Outcomes:** Traditionally, monitoring focused on measuring and evaluating the results of some system in operation on the dairy. Most of the measures used to monitor reproduction on dairies still fall into this class (e.g., days open, conceptions, pregnancies). These outcomes can be seen as the "objectives" of the system in operation, that is, its output. If the system is inadequate (in design or in operation or because

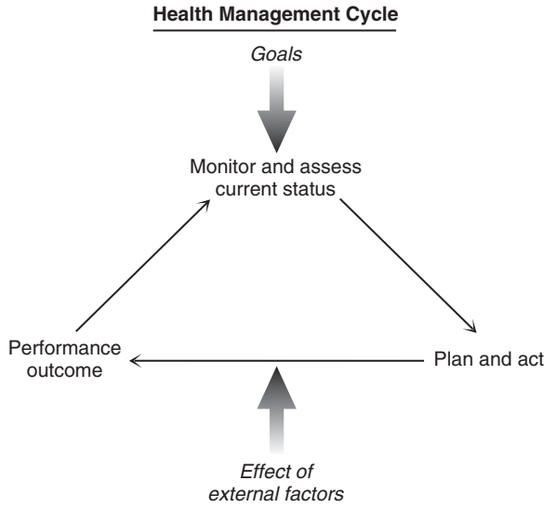


Fig. 61-1 Role of monitoring in farm management feedback cycle. (Adapted from Radostits O, Leslie K, Fetrow J: *Herd health: food animal production medicine,* 2nd ed. Philadelphia: WB Saunders, 1994.)

of unanticipated external influences), the objectives are not reached.

2. **Processes:** Monitoring can also be applied to systems in operation to discover whether the prescribed activities of the system are being done and being done properly. To be effective, this presupposes that a specific operating system is defined and the person(s) responsible are aware and properly trained. For example, in a dairy reproduction program based on estrus detection, are personnel actually taking the time to observe the breeding population of cows? Do they know what to watch for? Do they report observations to the right person in a timely manner? Are they accurate in their observations? Are enough cows bred within a specified period? The distinction between these two classes of monitoring sometimes is indistinct (e.g., are enough cows bred?), but the conceptual difference is valuable. Real beneficial changes on the dairy happen at the process level, and problems can often be most quickly detected as undesirable changes in processes. Economic impact largely happens at the outcome level. Process monitoring (oversight) is a major part of a manager's responsibilities and is key to assuring that the right things are done in the right way. Veterinary involvement in reproductive programs may include such process monitoring, either as a trouble-shooting activity or as part of the dairy's operating management. To be effective in the latter role, the veterinarian must be on the dairy on a consistent basis and sufficient time has to be committed to the task. Typically, such process monitoring by the veterinarian includes a training component.

As dairies become more rationalized and as science and process development advances, the connection between processes and outcomes becomes tighter and more predictable. As that development continues, more and more time and attention will be committed to process monitoring, with less focus on outcomes because they will follow more reliably (but not necessarily perfectly) from proper processes.

PITFALLS OF MONITORING PARAMETERS

The practitioner should be aware of several pitfalls in status and trend monitoring that can lead to inappropriate inaction (real problems escape notice) or inappropriate action (situations are misdiagnosed as problems and the wrong action is taken). Of the two, inappropriate inaction is probably the more common in reproductive production medicine and the more costly. The major pitfalls are as follows.

1. **Variation in methods of calculation:** There are many sources of reproductive indices and each has arrived at its own (often undocumented) approaches to calculating reproductive indices.[2] Recommendations regarding calculation have been put forth, but remain inconsistently implemented.[5] Furthermore, new approaches have become more appropriate since the last time a committee met to consider standardization. Care is necessary in interpreting numbers as presented, particularly when comparing results between various recording and summarizing programs.[2,5,6]
2. **The dangers of averages.** Reproductive herd records, and therefore reproductive herd monitoring, are rife with averages (means). Average days open, average services per conception, average annual culling rate as a percent of the herd (which is neither an average nor a rate)—these and others are used daily by veterinarians as they try to track the reproductive performance of their client herds. Averages measure one type of central tendency of a distribution of individual observations. Alone, they do not describe the spread of the distribution, nor do they call attention to failures of opportunities. Average days open, for example, can be the same in different herds, but with very different distributions. One herd can have a well-managed, tightly clustered distribution around the mean, while in another herd some cows might become pregnant very early while others are extremely delayed. Both herds might have the same average number of days open.

   Herd size can have a dramatic effect on the variation and computation of averages. In a 50-cow herd with 25 confirmed pregnant animals, a single cow with 350 days open increases the average days open of the currently pregnant cows by 10 days. If this cow is then sold, the average of the remaining 24 will drop by 10 days (example assumes average days open of 100 days). If the dairy farmer is unaware of this, false credit for a positive result may be given to an irrelevant intervention. Conversely, two animals conceiving

at 37 days would drop the average of the 27 pregnant animals by 5 days. At the other extreme of herd size, in very large herds with many cows contributing to the parameter, the average in any time period will tend to regress toward the long-term mean, obscuring problem cows that as individuals can be quite costly. Herd size effects cannot be avoided when averages are calculated; the practitioner must be aware of the possible pitfalls and use judgment when analyzing reproductive records.

3. **Percentages.** Percentages, like averages, can be misleading. In small herds or with small subsets of large herds (e.g., first-lactation animals bred by Joe Smith in June), one must be very careful not to overinterpret deviations from proportions used as standards. As an example, the 95% confidence interval (i.e., the true mean likely falls within this range) for services per conception when 13 pregnancies result from 40 breedings (a 33% conception rate) extends from 17% to 48%. One end of that range is a conception rate disaster, the other would be considered outstanding on modern commercial dairies. For many smaller Midwestern dairies, 40 breedings would take 6 months to accumulate, making tracking conception rates a problematic process. The practitioner must use judgment about when to intervene. Dairy managers are rarely willing to wait until something is statistically proved before they intervene. The burden of "proof" necessary to motivate management action can be quite different than the proof needed to establish scientific "truth." Not doing something when it is needed can be just as costly (or more so) than doing something unnecessary.
4. **Momentum.** Momentum in a parameter occurs whenever the events from the distant past are included in the current calculation of a parameter. For the parameter to change appreciably, given the weight added by events that are long past, then either the current status must change radically or significant time must pass. Either way, momentum tends to dampen change in a parameter, obscuring the actual status and recent trend. Both average days open and calving interval include a great deal of momentum.
5. **Lag.** Lag or delay occurs when the outcome of an event or intervention cannot be measured for some extended period of time. Lag is inherent, for example, in the calculation of conception rates, because conception (pregnancy) cannot be confirmed until at least several weeks after breeding. Lag becomes more severe when an inappropriate parameter is chosen to monitor a management program's results. An example might be the use of age at first calving to monitor the results of a prostaglandin synchronization program in heifers. The lag period is nearly a year before the effect of the program can be measured. A parameter such as the number (and identification) of heifers older than 13 months and not bred would have far less lag and thus would be more valuable as a monitoring tool for this prostaglandin program.
6. **Bias.** Bias can be introduced in many ways into the calculation of parameters that monitor status and trends on dairy farms. Bias exists when a systematic error is made in the selection of animals used in the calculation, the information used is incomplete or inaccurate, or the assumptions made about the biology are wrong. Bias distorts the parameter's true representation of reality. Some of the causes of such bias in reproductive parameters are as follows.
   - **Effect of culls and "do not breed" cows.** In many record systems, animals that have left the herd are not included in the calculation of some reproductive parameters. For example, the average days in milk at first breeding for the past 6 months should logically include any animal that was inseminated for the first time in the past 6 months, whether she is currently in the herd or is no longer present. Similarly, cows designated as "do not breed" cows may also be excluded. These animals are commonly those with the worst reproductive performance. Their exclusion from the data set would make the reported parameter better than reality in the herd.
   - **Presumption of reproductive outcome.** Reproductive calculations often may make optimistic assumptions about the outcome or status of some animals. These compromise-approaches to calculating parameters are legitimately necessary in some cases, but the practitioner needs to understand that the parameter may represent the best-case scenario. As an example, some record systems may assume that all cows that are bred and not checked for pregnancy are pregnant for the purpose of calculating average days open.
   - **Exclusion of subpopulations.** Some measures report on the performance of individuals with a positive (or otherwise known outcome), but ignore (or do not reflect) the current numbers of animals either pending status confirmation or past a management cutoff with no action. As an example, average days open in pregnant cows excludes cows from the calculation that are not yet confirmed pregnant. These may be the most important cows from a reproductive program perspective.
   - **Synchronization programs.** The routine use of prostaglandin or other hormone interventions for estrus induction, although often cost-effective management strategies, wreaks havoc with the calculation of many reproductive parameters. Apparent estrus detection rates are often calculated based on the assumption of 21-day estrous cycles. Inducing estrous cycles obviously makes this assumption false. Similarly, calculation of the interestrous interval and the expected pattern of estrous cyclicity are

severely confounded in herds in which estrus synchronization is used.
- **Use of bulls.** The calculated reproductive parameters can be severely distorted by the use of bull breeding, especially in herds in which the use of bulls is poorly recorded. For example, pregnancy to the bull may be calculated as resulting from an artificial insemination (AI) breeding, influencing the apparent services per conception rates.

Although the preceding list of pitfalls may seem long, it should not deter practitioners from working with records to analyze a herd's reproductive performance. Instead, knowing these pitfalls should lead to a healthy skepticism about the number presented, a determination to increase accuracy and completeness of the underlying data, and a fuller understanding of the conditions under which parameters may not truly represent herd status.

Inadequacies of the Calving Interval

The classic parameter for monitoring the status of a reproductive program has been calving interval. Although a short calving interval might be the *goal* of a reproductive program, it is totally inadequate as a *monitoring parameter.* Calving interval has severe momentum. It requires two consecutive calving dates, so on a herd calculation basis, events from as much as 2 years before still enter into the current calculation. Similarly, it suffers from severe lag, because the outcome from reproductive management efforts must wait through at least one entire gestation before the results can be calculated. Calving interval introduces bias by excluding populations (culled cows, first-lactation animals, animals pregnant but not yet calved, "do not breed" cows). Last, by being an average, calving interval has all of the computational pitfalls described for averages. Veterinarians, as a profession, must stop using calving interval as a monitoring parameter.

Terminology Issues

There are many terms used in describing reproductive programs, terms that have survived and become part of the everyday lexicon of dairying. Unfortunately, some of these terms are not consistent with standard meanings of words. Although it is likely that many terms will go on being used even though improper, it would serve the industry and profession if everyone could be a bit more precise in how words are used. Specifically, rate is a term often misapplied to reproductive parameters. A rate is formally a measure of an event or statistic *per time period*. Thus, miles per hour or deaths per 1000 cows per year are rates. Unfortunately, many parameters associated with reproduction are discussed as rates but in fact are not rates. For example, conception rate is not a rate (no dimension of time). Conception rate (pregnancies per insemination) is a risk (proportion of a population with some particular characteristic[7]). Older terms with widely accepted meanings are probably best left as is. New terms should be more carefully chosen.

GENERAL GOALS OF A REPRODUCTIVE PROGRAM

It has long been known that there is an important economic advantage to be gained by efficient reproduction in dairy herds.[8–12] The economic effects of a sound reproductive program include increased milk by returning cows sooner to the earlier, more profitable phase of their lactation, increased numbers of replacement heifers and bull calves born, reduced costs of reproductive disease and reduced costs from culling, reduced nonproductive days due to extended dry periods, and increased rate of genetic gain.

On a biologic basis, the goal of a reproductive health program on a commercial dairy can be summarized as follows: Throughout her herd life, a cow should calve without difficulty and deliver a live calf, experience little or no postpartum reproductive disease, begin to cycle soon after calving, be inseminated soon after the voluntary waiting period, conceive to a high genetics bull within an optimal time period (or conceive at the right age as a heifer), and carry each fetus to term. This is not to imply that the goal is elimination of all pathologic events; to do so would be biologically impossible and economically inefficient. Rather, the goal is to have a minimum of pathologic events and a maximum of productivity within the constraints of practical biologic and economic reality. This general goal can be subdivided into sections, as follows.

1. **Prompt rebreeding** (appropriate voluntary wait period). After calving, uterine involution should occur promptly and cows should be reproductively sound by 45 days in milk (DIM). Once past the farm's voluntary wait period (VWP; usually 45–60 days), cows should be seen or induced in estrus, be inseminated, and conceive in an efficient manner. If both estrus detection and conception rates are adequate, then only a small percentage of cows should have extended days open.
2. **Genetic return.** Genetic return on the investment in semen or bulls should be optimized. Some computer packages can calculate optimal bull profiles for selection for artificial insemination, given the farmer's goals for genetic gain and variability.[13] It is worth noting that the genetic return on the investment in semen occurs only if the insemination results in a live *female* calf that subsequently conceives, calves, and has a productive lactation. Genetic improvement on a dairy farm is a long-term and somewhat risky investment. Assuming a 40% conception rate, 44% of females not born co-twin to a bull, a 10% abortion rate after pregnancy diagnosis, and a 20% loss of replacements from parturition until the end of a productive first lactation (includes stillbirths), only 13% of inseminations actually return any appreciable value in genetic gain to the producer. This means it takes at least 7 straws of semen just to produce a first lactation animal. On many farms, they need to purchase

well over 10 straws to produce a complete first lactation.

3. **Pregnancy wastage.** Pregnancy wastage should be at a practical, economic minimum (early embryonic death, abortion, stillbirths). Recent adoption of pregnancy diagnostic tests that can detect conception at an earlier stage is changing how pregnancy wastage is defined and the proportion of lost "conceptions" that are expected.
4. **Disease.** Peripartum disease morbidity should be minimized, enhancing animal welfare, avoiding impacts on later reproductive performance, lessening loss of cows either by death or culling, and minimizing therapy and labor costs.

MONITORING STATUS AND HERD REPRODUCTIVE TRENDS

Many parameters are used to monitor reproductive status and trends on the dairy farm. Some of these goals are shown in Table 61-1. For the most part, these are the traditional monitoring parameters for dairy reproduction. These herd goal levels must be applied with caution and may not be the appropriate alerting levels for management intervention on an individual cow basis. Several reviews of these parameters and their application have been written, so what follows is only a brief outline of the major parameters.[5,14–20]

Overall Reproductive Efficiency

Herd Distribution of Cows by Reproductive Status

Perhaps the best starting place for evaluating a dairy's reproductive status is simply a breakdown of cows by reproductive status and lactation (see Table 61-1[21]). Cows are split into the following groups:

1. *"Do not breed" cows:* cows for which management has decided to stop inseminations
2. *Fresh cows:* typically cows less than the voluntary wait period; on some dairies those cows not yet confirmed to have completed their normal involution post partum
3. *Cows OK to breed and known to be open:* includes cows previously bred but determined to be open
4. Cows bred and awaiting pregnancy diagnosis
5. Pregnant cows still milking
6. Dry (nonlactating), pregnant cows
7. Animals that have exited the dairy in the time frame of interest (whether sold or died)

Note that the principal focus of a dairy's reproductive program should be on cows in preceding groups 2 and 3, that is, those that can either be seen in estrus or synchronized to come into estrus and be inseminated and those transition cows that need careful attention, with lesser focus on early postpartum cows for uterine pathology (part of group 2) and bred cows that need a pregnancy diagnosis (part of group 4). This breakdown of cows in the herd may help focus management attention on those cows that can be usefully affected by management action.

Pregnancy Risk (Pregnancy Rate)

Pregnancy risk is probably the single most telling parameter to evaluate the reproductive performance of a dairy (although pregnancy risk alone is not sufficient, as noted earlier). Pregnancy risk is the proportion of open cows that become pregnant during a specified period of time. It is the proportion of cows that make the transition from open to pregnant (as a percentage of eligible cows) over a 21-day period. Thus, it estimates the "risk" that an eligible open cow will become pregnant on the dairy in the next 21 days. The definition of pregnancy risk is the total number of cows that become pregnant during a period of time (typically 21 days) out of the total number of cows eligible to become pregnant during the same period.

To be eligible, each cow should meet the following criteria:

- The cow should be past the voluntary wait period at the beginning of the period.
- The cow is known to be open at the start of the period.

TABLE **61-1**

Breakdown of Cows in a Dairy Herd by Reproductive Status (RPRO)

COMMAND: SUM BY RPRO LCTGP FOR LACT>0 RC>0\B

RPRO	%COW	#COW	LCTGP=1	LCTGP=2	LCTGP=3
NO BRED	0	3	0	1	2
OK/OPEN	17	158	54	46	58
BRED	19	182	81	48	53
PREG	27	256	24	78	54
DRY	6	64	25	17	22
SLD/DIE	27	252	49	63	140
Total	96	915	233	253	329

NO BRED, do not breed cows; fresh, cows not ready to breed; OK/OPEN, cows available to breed; BRED, cows bred, not checked for pregnancy; PREG, pregnant, milking cows; DRY, pregnant, dry cows; SLD/DIE, sold or dead cows. LCTGP, lactation groups are first, second, and third or later lactations.

- The cow is not flagged as a "do not breed" cow in the period.
- The outcome of any insemination during the period is known—pregnant or open.
- Additionally, if both AI and bull pens are used on the dairy, the AI pregnancy risk should not include any animal that was not inseminated in the AI pen but was moved into the bull pen during the period.
- **Note:** for computational purposes, cows that meet these criteria for at least half of the 21-day interval are eligible.

Pregnancy risk has been estimated by multiplying estrus detection rate (or insemination risk) times the conception rate in a herd, but this estimate is fraught with potential errors. Estrus detection rate may not include cows not yet bred, estrus events may be detected but the cow not inseminated,, and estrus may be recorded more than once in a 21-day period. This may bias the estimate of estrus detection in terms of a cow's single chance of getting pregnant in a 21-day period. Conception rate calculations may only include pregnant cows, and again, cows can be bred more than once in a 21-day period. Finally, the time periods over which estrus and conception rates are calculated are often not the same. It is far better that pregnancy risk be calculated directly from cow level data, not computed from estrus and conception rate parameters.

Pregnancy risks may be calculated for a single cycle, but one must be careful not to extrapolate too freely from single cycle results to overall herd performance. Particularly for programs that synchronize estrus, the first cycle of a program may achieve relatively high pregnancy risks (essentially equal to conception rate as all cows are inseminated), but the following cycle may experience much lower pregnancy risks, as cows awaiting pregnancy examination are not detected as they return to estrus. In synchronization programs in which cows have different risks of pregnancy in alternating cycles, any calculation of pregnancy risk across an odd number of cycles will distort the herd's real reproductive performance. Similarly, one should be careful of only calculating pregnancy risk for a set number of days of lactation which may only include an odd number of cycles.

Pregnancy risks have the advantage that the only lag in their calculation is the period until pregnancy status can be determined after breeding. Because all eligible cows are included, bias is not introduced in the parameter. If the risk is calculated for each successive 21-day period, there is little momentum in each number and trends in the parameter can be observed (Table 61-2). Pregnancy risks vary widely across dairies, with typical average pregnancy risks of about 12% to 14% (Fig. 61-2[21]). If a dairy could reliably and accurately submit 60% of eligible cows for insemination at least once in a 21-day period and achieve 40% conception for animals submitted for insemination in this 21-day period, the pregnancy risk would be 24%. Clearly, there is a great deal of opportunity to improve overall reproductive performance on most dairies.

The data that were used in generating Figure 61-2 were mostly based on pregnancy confirmation by rectal palpation, typically at day 35 after breeding or later. These

TABLE **61-2**

Example of Pregnancy Risk Determination for a Dairy Herd

				COMMAND: BREDSUM\E			
Date	**Ht**	**Elig**	**Heat**	**Pct Pg**	**Elig**	**Preg Pct**	**Aborts**
2/27/03	175	102	58	172	36	21	6
3/20/03	165	81	49	164	26	16	1
4/10/03	182	94	52	177	33	19	2
5/01/03	171	111	65	167	50	30	8
5/22/03	138	58	42	136	22	16	4
6/12/03	122	75	61	121	25	21	0
7/03/03	113	53	47	112	15	13	2
7/24/03	138	75	54	138	18	13	5
8/14/03	152	87	57	151	21	14	7
9/04/03	148	82	55	146	23	16	1
9/25/03	174	100	57	172	35	20	6
10/16/03	171	91	53	170	29	17	3
11/06/03	184	115	62	183	34	19	3
11/27/03	168	72	43	166	19	11	2
12/18/03	182	88	48	176	25	14	4
1/08/04	192	113	59	188	43	23	2
1/29/04	179	89	50	0	0	0	0 no preg status
2/19/04	152	105	69	0	0	0	0 no preg status
Total	2575	1397	54	2539	454	18	56

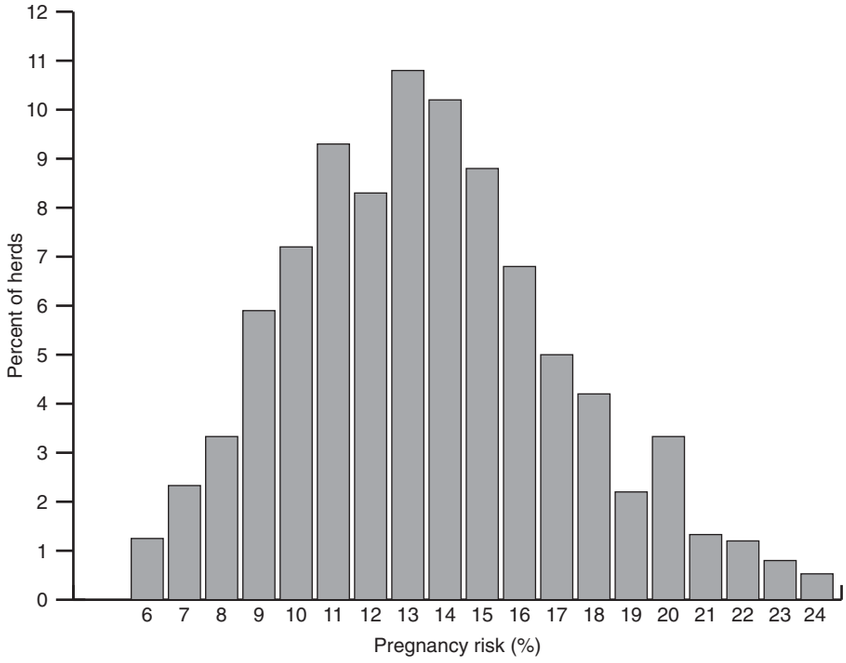


Fig. 61-2 Pregnancy risks: Minnesota dairies: risk that a pregnancy eligible cow will become pregnant in a 21-day period. (Data provided by Dr. Steve Stewart based on 1532 Minnesota DHIA herds, 2003.)

are the numbers routinely used by dairy veterinarians and for which the profession has a sense for what is acceptable and what is not. The advent of earlier pregnancy diagnosis (e.g., ultrasound and possibly immunologic tests) will tend to shift expectations. Ultrasound at day 26 will detect pregnancies that may no longer be there at day 42 after insemination. If these earlier "pregnancies" are considered real, the computational result will be higher pregnancy risks, but also higher abortion risks, as some of those early embryos are lost. There is not a consensus within the industry yet about how to handle this issue. Perhaps agreement could be reached that would continue to refer to pregnancy losses after pregnancy confirmation at 45 days (or 60) of gestation as abortions, and losses earlier as embryonic deaths. However, most dairies cannot reliably identify when a cow actually aborted.

Number of Pregnancies per Time Period

A pragmatic, but not particularly diagnostic approach to monitoring reproductive function on a dairy is to simply monitor the number of confirmed pregnancies per period of time. The logic is approximately as follows: If a dairy intends to calve 120 animals per year (as an example), then on average it must achieve somewhat more than 10 pregnancies per month in cows and heifers (more than 10 to allow for some abortions and cow culling) in order to have enough calvings 9 months hence. Given the average conception rate, one can also use this approach to monitor the number of breedings needed each month as well. This approach may be crudely useful for a quick managerial scan of reproduction (very little lag, no momentum), but it cannot detect the sector of the reproductive program in which a breakdown may have occurred.

Days Open

Better described as the calving-to-conception interval, in the past this was the most widely used parameter to assess "overall" reproductive performance in a herd.[22] As calculated by many DHIA record programs, it tends to estimate the minimum projected days open, and in some cases is named just that. Calculated on an annual basis, days open has significant momentum and lag and is distorted by exclusions, culling, "do not breed" cows, and assumed outcomes. Different record systems deal with these issues in different ways.[22] Nonetheless, it is a readily available and understandable parameter and so remains in widespread use. In addition to the difficulties inherent in calculating average days open, depending too much on this single number may mask serious reproductive inefficiency from a wide distribution of individual cow performance. Suffice it to say, although some veterinarians will continue to use average days open as a historical assessment of reproductive efficiency, prudent practitioners will do so with care.

Calving Interval

Although maintaining a short calving interval is the conceptual goal of reproductive management, the parameter itself is fraught with problems. As noted earlier, calculating an actual calving interval requires that a cow has calved twice. The parameter has severe momentum and excludes first-lactation animals and culled cows. It is the weakest monitor of a herd's reproductive performance, and should not be used.

Survival Curves

Survival curves are the graphic presentation of the change in a population from one "state" to another over time,

for example, from being open to being pregnant or being not yet bred to bred for the first time. Ideally these curves could provide some information about the trajectory of transition, about what proportion of the population is pregnant or bred by a given time, and about what portion ultimately does not become pregnant or get bred. The slope of the survival curve is *not* the rate of conversion (the "risk") because the denominator population changes at the same time as the numerator changes. There are significant and troubling problems in the construction of these curves in all but deliberate prospective studies (e.g., in defining what the population at "risk" is for a survival curve, what to do with a cow bred 34 days ago but not declared pregnant, what to do with a cow that was pregnant, lost the fetus and is pregnant again). Survival curves are perhaps most useful when two different beginning populations are compared on the same curve. Differences in the experiences of the two populations are often rapidly apparent but may be confounded by differing time periods for the two populations, culling rates in early lactation, etc. (Fig. 61-3[21]). Although survival analysis is a valuable research evaluation tool, it is a problematic herd monitoring tool.

Breaking Days Open into Components

In a pivotal 1975 paper, Barr[23] laid out a conceptual framework for allocating the total days open to several components. That conceptual framework remains a cornerstone of the production medicine approach to addressing reproductive efficiency on dairy farms. As stated by Ferguson and Galligan, "most variations in herd performance result from pathology of management and not pathophysiology."[24,25] Barr's conceptual framework allows the practitioner to dissect the influences of management to localize the subsystem that is responsible for poor reproductive performance. Figure 61-4 provides a schema for this framework, as well as identifying typical problems by subsystem.

Barr's concept is that days open (in normal, fertile cows) comes from four sources:

1. Farm policy for the voluntary wait period (VWP).
2. One half of an estrous cycle past the VWP for the first estrus to occur. Some cows may be in estrus the day after the VWP and others 20 days after the VWP, but on average, it will take one half of a cycle. This is physiologically dictated, in the absence of synchronization programs.
3. Failure to detect estrus. Again, each missed estrus requires another 21 days of waiting (in the absence of synchronization programs).
4. Failure to conceive when bred. Each failure to conceive adds another 21-day estrous cycle to the tally of days open.

The effects of estrus detection and conception rate are not independent. For example, if it takes two services per conception and if every other estrus goes undetected,

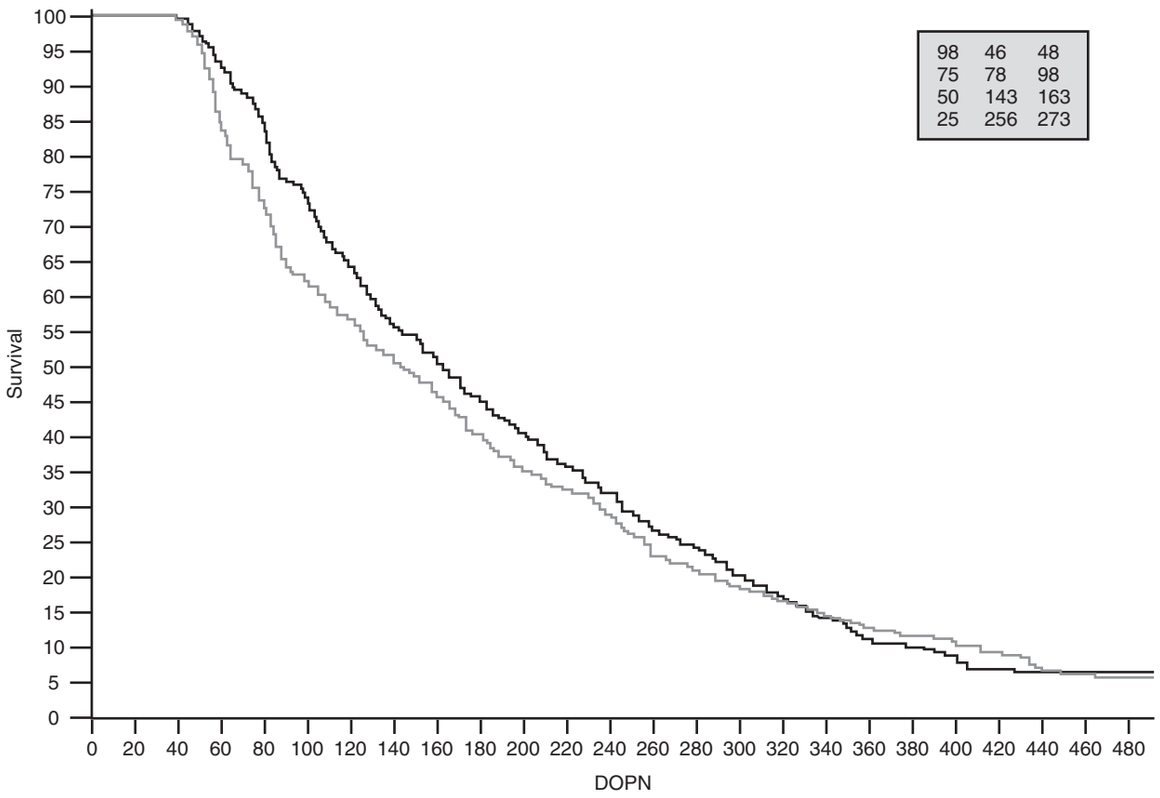


Fig. 61-3 Survival curve for days open for first lactation with second and older lactation.

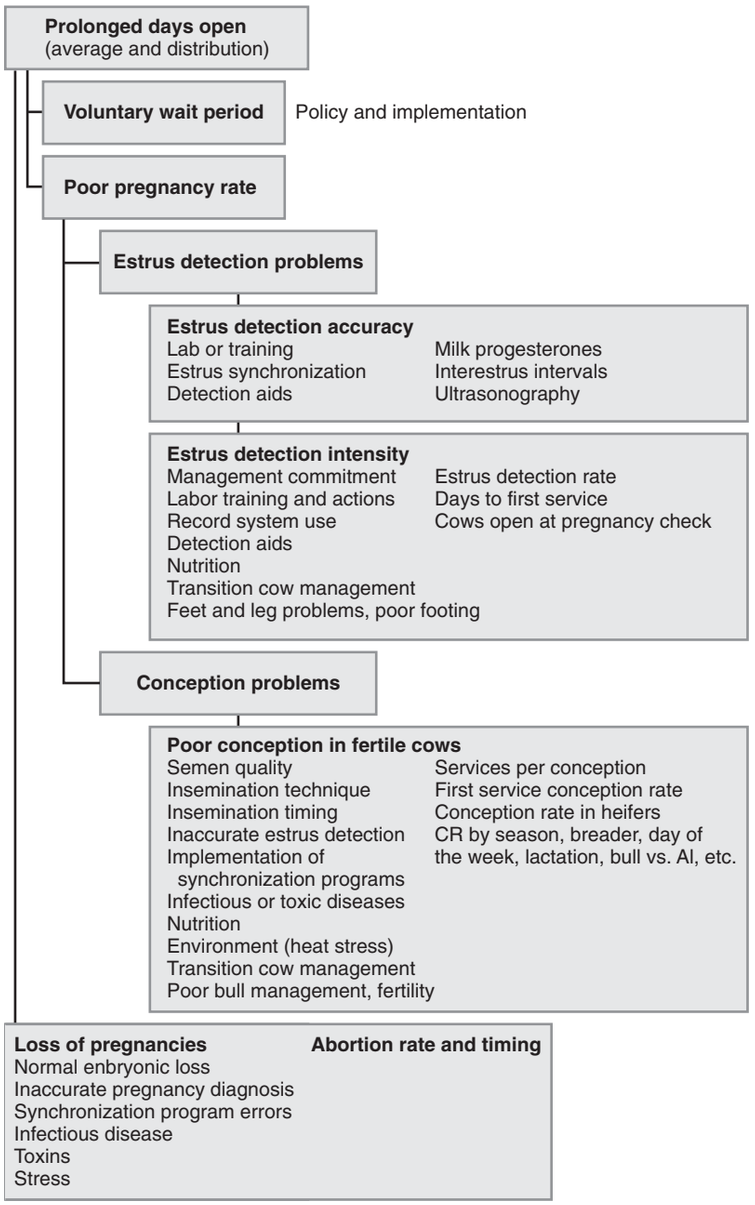


Fig. 61-4 Conceptual framework for evaluating dairy herd reproduction. (Adapted from Radostits D, Leslie K, Fetrow J, eds: *Herd Health: Food Animal Production Medicine*, ed 2, Philadelphia, WB Saunders, 1994.)

then it will take four estrous cycles to detect the two estruses that are needed to achieve two breedings and one pregnancy.

Consider the example of a herd with the following reproductive performance: 50-day VWP, 33% conception, 50% of estruses detected. (DIM = days in milk, or days since calving):

50 DIM: voluntary wait period
60 DIM: average cow has her first estrus in the breeding cycle, which goes undetected
81 DIM: second estrus, detected, inseminated, does not conceive
102 DIM: third estrus, not detected
123 DIM: fourth estrus, detected, inseminated, does not conceive
144 DIM: fifth estrus, not detected
165 DIM: sixth estrus, detected, inseminated, conceives

With no disease, no abortion, and normal management, the average cow has 165 days open. A tally of the 165 days by cause shows:

50 days: VWP
10 days: one half of an estrous cycle
42 days: failure to conceive on the two unsuccessful breedings
63 days: failure to detect three estruses

165 days total

This estimate of 165 days is based on performance in conception and estrus detection that is better than the average U.S. herd (note: this example would have a pregnancy risk of 17%), but it also assumes that all cows in the herd are fertile and are bred until they finally conceive. In reality, dairy managers give up at some point trying to get the final few percentages of cows pregnant and those cows are culled. As a practical example, given the aforementioned assumptions and accepting that no cow will be bred past 300 days in milk, the result would be approximately 134 days open for the average pregnant cow in the herd and roughly 11% of cows culled as not pregnant (Table 61-3[26]). These numbers do not account for the effects of abortion. This means that more than half of all herds are doing more poorly than 135 days open (accepting a 10% culling proportion for open cows), many substantially so. For most herds, achieving the oft extolled average days open number of 115 days (a 13-month calving interval) is an unreasonable starting goal under traditional management. It should be remembered, too, that in this example herd case, 11% of cows remain open after 300 DIM, in the absence of any reproductive disease.

A critique of Barr's concept lies in the assumption of no reproductive disease. The issues of infertile cows, chronic infections, and scarring of the reproductive tract should be taken into account. Although certainly operative on an individual cow basis, the effect of disease (absent herd level nutritional, toxic, venereal or contagious reproductive pathogens) seems to be small in most herds. In particular, there seems to be little reproductive "memory" from one lactation to the next, at least for cows that remain in the herd for a subsequent lactation. Current herd average days open seems to be the best predictor for an individual cow's days open, if that prediction must be made near the time she calves. When studied across a large population, a cow's reproductive history in the previous lactation had little or no effect on her reproductive performance in the current lactation.

Given the conceptual breakdown of the source of days open in a herd, the question is how one can monitor each of these critical controllable components.

Voluntary Wait Period

The obvious first step to determining the dairy's policy regarding voluntary wait period (VWP) is to ask. Unfortunately, stated policy and actual behavior are often not the same. The minimum wait period can be determined by finding the unusual cow with the fewest DIM at first breeding, but this is seldom a reasonable description of true management behavior of the herd. An estimate of actual VWP can be better derived from the DIM, where first breedings begin to cluster. Scatter plot distributions of days in milk at first breeding will give an impression of what is actually happening (Fig. 61-5[21]).

These types of graphic displays have the advantage of little or no lag or momentum. They are sensitive to the herd's status and help identify individuals with unacceptable performance. They seldom fail to illustrate real problems, although they may flag problems in individuals that are not indicative of a general herd mismanagement problem. They also allow action to proceed

TABLE 61-3

Example of a Spreadsheet Calculation of the Number of Cows Still Open and the Average Days Open by Estrous Cycle in Dairy Cows*

Cycle	Days in Milk	Cows Open	Number of New Pregnancies	Number Still Open†	Herd Average Days Open in Pregnant Cows
1	60	100	17	84	60
2	81	84	14	70	70
3	102	71	12	58	78
4	123	58	10	49	87
5	144	49	8	41	95
6	165	41	7	34	102
7	186	34	6	28	108
8	207	28	5	24	114
9	228	24	4	20	120
10	249	20	3	16	125
11	270	16	3	14	129
12	291	14	2	11	134
13	312	11	2	10	137
14	333	10	2	8	141
15	354	8	1	7	144
16	375	7	1	6	146
17	396	6	1	5	149

From John Fetrow, VMD.

*This dairy reproduction model assumes that only one estrus and one insemination per cycle. It does not account for abortions, and it assumes all cows are fertile.

†Reproductive culls if breeding stops at this point.

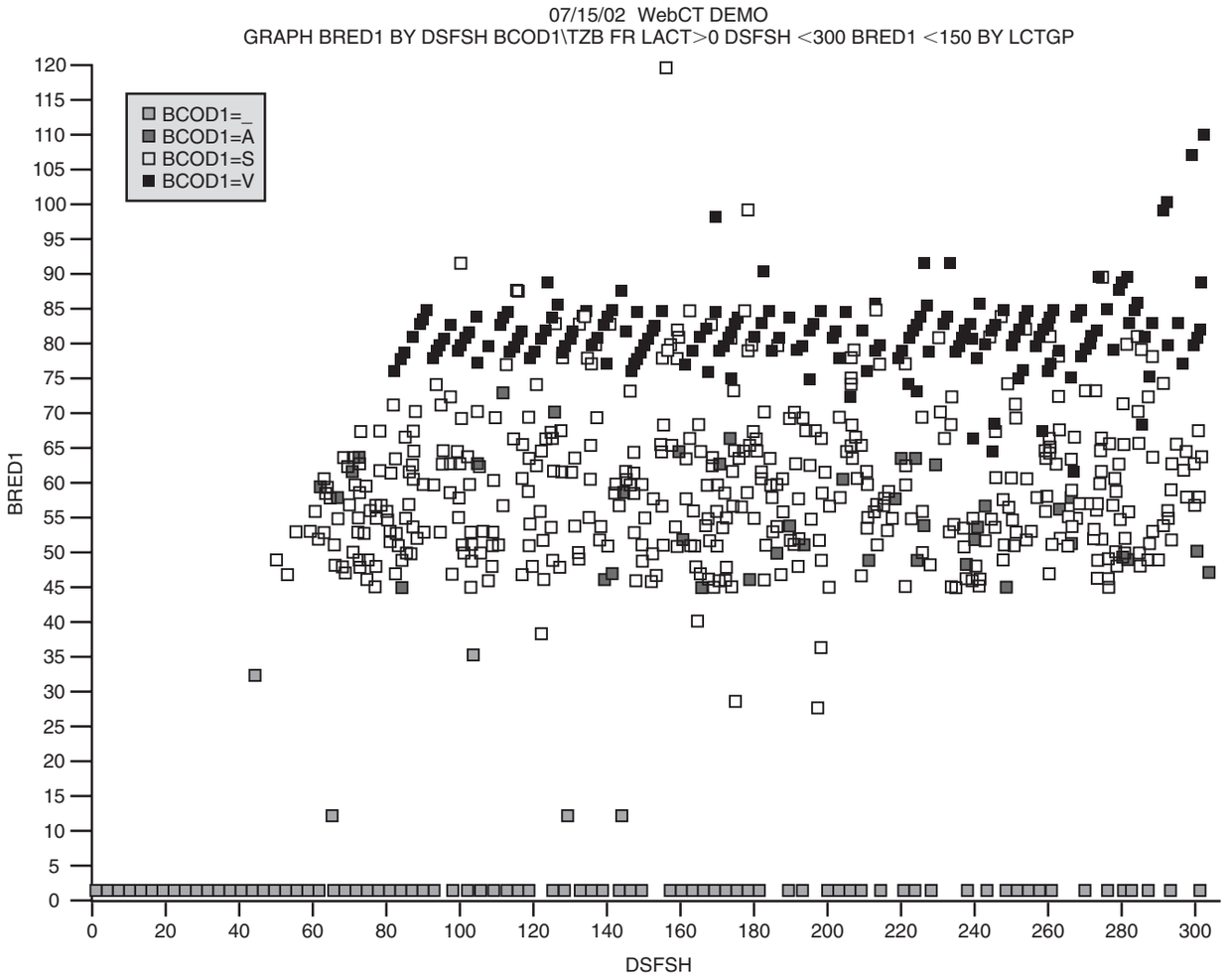


Fig. 61-5 Scatter graph of days at first breeding (BRED1) by current days in milk. This graph provides a reliable impression of the dairy's voluntary wait period before starting routine breeding (in this example about 50 days in milk). Note the use of routine synchronization in cows not bred by 75 days in milk.

without the need for precise quantification of overall herd parameters. Most important, they typically initiate a discussion.

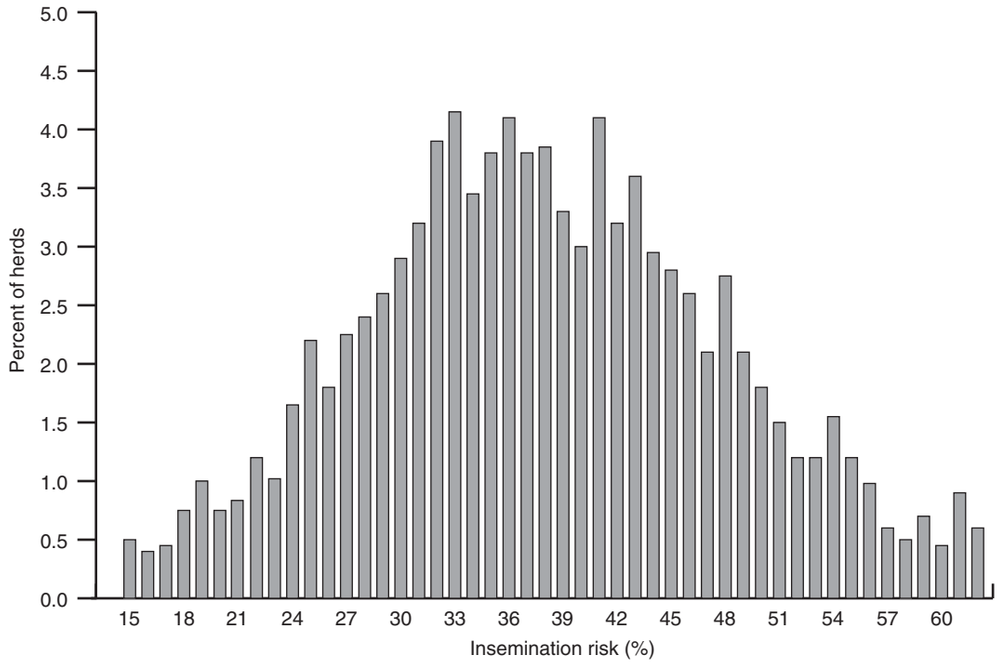
Estrus Detection

The two components of estrus detection are intensity (what proportion of cows are seen in estrus?) and accuracy (of those identified in estrus, what proportion really is in estrus?).

Estrus detection intensity. Percentage of estruses detected (estrus detection rate; actually a risk) is the usual parameter used to monitor estrus detection intensity. It is calculated based on the number of estruses detected over a period of time divided by the expected number of estruses in the breeding population (see Table 61-2[21]). Its calculation can have bias, depending on how cows are qualified as being eligible to come into estrus; it tells nothing about accuracy and is confounded by the use of synchronization programs.

Insemination risk is the chance that a cow will be inseminated at least once within a defined 21-day period. The difference between estrus detection rate and insemination risk lies in the point of view of the evaluation. Estrus detection rate measures how well the dairy identifies or induces cows to be in estrus, that is, the focus is on the cow and her behavior, heat detection aids, and labor on the dairy. Thus, a detected but noninseminated estrus counts in the calculation. In contrast, insemination risk focuses more on the herd dynamic: how effectively management is acting to get cows inseminated in a 21-day period. With insemination risk, cows bred twice in a 21-day period serve no better purpose than cows bred once, and cows detected in estrus but not inseminated do not count in the calculation. Insemination risk varies widely across herds, with the average rate centered at around 35% (Fig. 61-6).

Days to first estrus or days to first breeding is an indirect measure of prebreeding estrus detection intensity. If



Minnesota DHIA Data
1532 Herds Nov&Dec2003

	Ins_Risk	Preg_Risk	Conc_Risk
Mean	38.06%	13.92%	37.66%
Median	37.55%	13.56%	36.65%

Data provided by Dr. Steve Stewart based on 1,532 Minnesota DHIA herds, November and December 2003

Fig. 61-6 Insemination risk in Minnesota DHIA herds: risk that an insemination eligible cow will be inseminated at least once in a 21 day period. (Data provided by Dr. Steve Stewart based on 1,532 Minnesota DHIA herds, November and December 2003.)

the average is near to the VWP policy of the dairy (within 18 days or so in the absence of a synchronization program), then estrus detection intensity is probably acceptable. If the gap is longer, either estrus detection intensity is low, or the actual VWP is not the same as stated policy. Remember that this is a average, and calculation may suffer from lag, momentum, and bias. The scatter graph mentioned earlier will be far more useful.

The number of cows pregnant at pregnancy examination is a more indirect measure of estrus detection intensity. The logic of expecting more than about 80% of cows to be pregnant when examined is that if estrus detection is intense, then most cows that did not conceive when bred will be detected in estrus before pregnancy examination, leaving mostly pregnant cows to be examined. However, the expected percentage varies, depending on how long after breeding cows are examined for pregnancy, and there are formulae to calculate an estimate of estrus detection rate.[27] This measure of estrus detection intensity is confounded by conception rate as well; high proportions of open cows found at the pregnancy check can result from very poor conception as well as from poor estrus detection.[27]

A form of Q Sum graph can also be used to assess estrus detection intensity (Table 61-4[21]). The computer calculates each expected estrus for each cow, typically assuming a first estrus at day 50 and a 21-day estrous cycle (and adjusting for prostaglandin use, if recorded). Given the expected estrus, the program then records the actual outcome and displays the result. In the graph in Table 61-4, O would mean bred and open, P would mean bred and known pregnant, B would mean bred and outcome unknown, and M would mean estrus missed. If an expected estrus is detected, the observation moves one character to the right. If missed, the observation moves one character to the left. For a 50% estrus detection rate, the line of observations would tend to fall vertically on the page. Although crude in some ways, this sort of graphic approach is a valuable monitoring, educational, and motivational tool. Because the data are sorted on a chronologic basis by day, Q Sum graphs are much less useful in large herds than in smaller herds.

Estrus detection accuracy

Milk progesterone. Cows truly in estrus should have low milk progesterone concentration. For reliable interpretation, 15 to 20 cows should be sampled, with milk taken at the same time as insemination or the first milking after insemination. Samples should be frozen until a cohort has been collected for testing. Care must be taken to conduct the tests properly. In practical application, fewer

TABLE **61-4**

Q-Sum Graph of Estrus Detection

			COMMAND: BREDSUM\H HEAT DETECTION:	
Cow	**Date**	**Lact**	**Dim**	----------0++++++++++++++++++++++++
112	11/3	6	105	B
21	11/4	2	134	M
52	11/4	4	113	M
119	11/5	1	230	M
16	11/7	3	511	M
9	11/8	1	134	M
45	11/9	4	50	M
77	11/10	1	155	M
22	11/11	1	222	B
61	11/12	1	86	M
126	11/12	2	134	M
90	11/12	4	263	O
65	11/12	7	245	B
15	11/12	8	105	B
205	11/13	1	180	O
29	11/13	2	315	M
70	11/13	2	455	M
14	11/14	6	117	M
69	11/17	2	128	M
53	11/19	2	92	M
3	11/19	3	128	M
73	11/19	3	128	M
30	11/19	4	128	M
68	11/21	2	113	M
21	11/25	2	155	M
76	11/25	2	513	B
52	11/25	4	134	M
119	11/26	1	251	B
9	11/29	1	155	M
107	11/29	3	50	M
45	11/30	4	71	M
16	11/30	3	534	B
77	12/1	1	176	M
61	12/3	1	107	B
126	12/3	2	155	M
33	12/4	3	50	M

Estrus events are projected for each eligible cow. If an estrus is detected, the graph moves to the right (B means bred; O means bred but open). If the projected estrus is missed, the graph moves to the left (M means missed). In this example, estrus detection efficiency is less than 50% (a vertical progression downward is 50%).

than 10% of cows bred should have luteal levels of milk progesterone at the time of breeding.[28]

Interestrous intervals. With classic breeding programs, an argument can be made that estrus detection accuracy can be evaluated by monitoring the interval between breedings for cows (Table 61-5[21]). In theory, the intervals should be some integer multiple of 19 to 23 days; that is, they should follow a normal estrous cycle. The widespread use of synchronization programs on dairies often renders this approach unworkable.

Conception Rates

Various measures of conception efficiency are available: services per conception in pregnant cows (ignores repeat breeders), in all bred cows, stratified by lactation, by season, by artificial insemination technician, and so on. These analyses depend on accurate recording of the necessary information by the breeding staff of the dairy. Conception rates (again, really risks) on dairies range widely, with the median conception rate in a large sample of Minnesota dairies at 38% (Fig. 61-7). Table 61-6 shows one such stratification, in this case for conception by breeding type.[21] By their nature, conception parameters always have lag and momentum. Each parameter provides a window onto conception efficiency, and a combination of parameters can focus on the possible problem area if there is a serious conception problem in the herd. Q sum graphs can also be applied to conception in smaller herds, with the graph moving right when a breeding is successful and left when a breeding is unsuccessful.

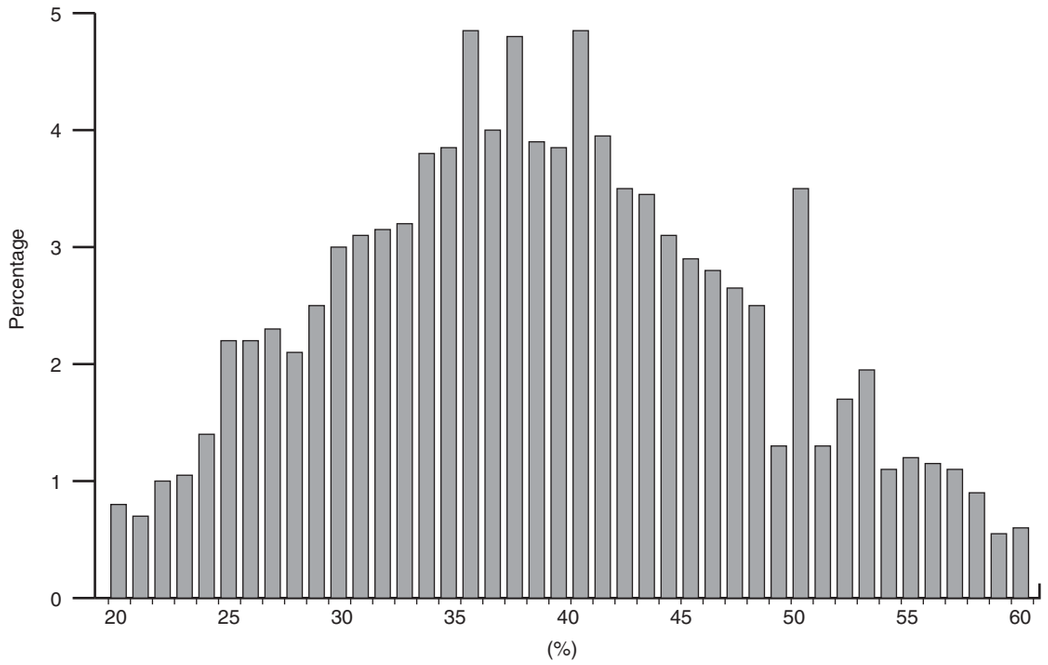
TABLE **61-5**

Chart of Interestrous Intervals on a Dairy

COMMAND: BREDSUM \ID180 FOR LACT>0

Heat Interval	%Conc	#Preg	#Open	Other	Total	%Tot	SPC
1–3 days	25	2	6	1	9	1	4.0
4–17 days	21	5	18	2	25	4	4.6
18–24 days	45	39	46	7	92	16	2.2
25–35 days	45	28	34	1	63	11	2.2
36–48 days	32	61	127	11	199	36	3.1
Over 48 days	35	54	100	8	162	29	2.9
Totals	36	189	331	30	550	100	2.8

Ideally, most inseminations should fall between days 19–23, or multiples thereof. This approach provides one measure of estrus detection accuracy. This is distinctly confounded by the use of synchronization programs that can return cows to estrus at other than 21-day intervals. Conc, conception rate; SPC, services per conception.



DC305 Conception *Rates: MN DHIA Jan–Mar 2004*
38.9 Mean (20–60 CR)
38.0 Median (20–60 CR)

Fig. 61-7 Conception rates in Minnesota DHIA herds 2004. (Data provided by Dr. Steve Stewart based on 2,193 Minnesota DHIA herds, 2004; only herds with conception rates of 20–60 percent are included.)

Bull Performance

There are many aspects involved in bull performance on dairies. Typically bulls are used as "clean up" breedings, with cows not pregnant to a series of AI inseminations turned into bull pens for breeding. Bulls are often poorly managed on dairies (poor feet, overused, not rested, etc.) and the result is that bull performance is commonly far below optimum. Dairymen may have a belief that the bull is naturally more efficient at getting cows pregnant than AI programs, but hard data often show this is not the case. If dairies reliably designate bull pens, record when cows are moved into those pens, and confirm pregnancy such that AI and bull pregnancies are distinguished, then pregnancy risks for bulls can be calculated in the same way as AI breedings (Table 61-7). It is not

TABLE **61-6**

Example Breakdown of Conception Rates for a Dairy

COMMAND: BREDSUM\O

Breeding	Code	%Conc	#Preg	#Open	Other	Total %	Tot SPC
Luteal heat	34	51	97	2	150	8	2.9
Ov-Synch heat	29	245	580	36	861	48	3.4
Standing heat	41	300	425	47	772	43	2.4
Totals	35	596	1102	85	1783	100	2.8

In this case the breakdown is by type of breeding, but many other breakdowns are possible and may be useful in characterizing conception problems (e.g., by day of week, by technician, by season, by insemination number). Conc, conception rate; SPC, services per conception.

TABLE **61-7**

Pregnancy Risk Performance by Clean-up Bulls on a Dairy

COMMAND: BREDSUM \U

Date	Ht Elig	Heat	Pct	Pg Elig	Preg	Pct	Aborts
2/07/02	13	2	15	13	1	8	0
2/28/02	14	1	7	14	1	7	0
3/21/02	11	3	27	11	2	18	0
4/11/02	18	4	22	18	4	22	1
5/02/02	12	1	8	12	1	8	0
5/23/02	11	1	9	11	1	9	0
6/13/02	17	2	12	17	0	0	0
7/04/02	33	0	0	33	0	0	0
7/25/02	36	7	19	35	4	11	0
8/15/02	28	1	4	28	1	4	0
9/05/02	29	4	14	29	3	10	0
9/26/02	28	4	14	28	2	7	0
10/17/02	29	5	17	29	3	10	0
11/07/02	26	10	38	25	4	16	0
11/28/02	26	3	12	26	0	0	0
12/19/02	27	3	11	26	1	4	0
Total	358	51	14	355	28	8	1

The bull pregnancy risk (8%) was substantially below the pregnancy risk for AI breedings in this herd.

uncommon for bull pregnancy risks to be poorer than AI rates in the same herd.

Reproductive Disease

Monitoring the incidence of reproductive disease can be useful as an indicator of underlying problems with management. It is important that the dairy settle on a consistent case definition so that everyone involved understands what and when to record as an event. Typically, retained placentas, metritis, dystocia, and stillbirths are monitored as indicators of dry cow and transition feeding program management (particularly mineral and energy balances), calving area hygiene, and calving supervision. Cystic ovarian disease incidence may be an indicator of early postpartum energy status, but care should be exercised to distinguish true high incidence from overzealous diagnosis. As a rough guide, no reproductive disease should have an incidence higher than 10% of cows per lactation, although this may be too stringent a standard for metritis, depending on the case definition used. The trend in incidence over time is a more useful indicator of problems than the specific level in any period.

Abortion

There are two scenarios in which abortion becomes a herd problem. The first is a herd in which there is an increased incidence of abortion over a long time frame (endemic abortion). The second is when a clustering of abortions occurs in a short time frame (an abortion outbreak). Determining when a herd has had an "abnormal" number of abortions is a difficult and controversial question and requires knowledge of what a "normal" abortion rate is and what level above the normal rate is acceptable to that particular dairy. Estimates of abortion are also

confounded by the time of pregnancy diagnosis. Increased use of ultrasound for early pregnancy diagnosis will tend to elevate early pregnancy loss estimates compared to diagnosis based on rectal palpation 10 to 14 days later. The stages of pregnancy may be conveniently broken down into specific stages:

Gestation days 1 to 42: embryonic period (growth of embryo, no or limited placental attachment)
Gestation days 42 to 120: early fetal stage (placental attachment having taken place by about 45 days,[29] no or limited fetal immune function)
Gestation days 120 to 180: middle fetal stage (development of immune function)
Gestation days 180 to 260: late fetal stage (beginning of rapid increase of fetal weight)
Gestation days 260 to 280: premature birth stage (calf may be viable if born; stillbirth common if delivered prematurely)[30]

Estimates of the normal abortion rate for dairies range from 0.4% to about 10%.[31,32] Findings as low as 0.4% almost certainly reflect failure of diagnosis. One large study in 10 northwestern U.S. dairies found that 11% (range 7.6–13% by herd) of pregnancies diagnosed by rectal palpation after 31 days' gestation were lost between day 42 and day 260, during the fetal stage.[33] The range of reported abortion rates may be due to the definition of what constitutes an abortion (time in gestation), completeness of detection of lost pregnancies, the accuracy of the original diagnosis of pregnancy, and the population of cows that are used as the denominator in the calculation. Most loss of pregnancy does not lead to an observation of the aborted fetus or membranes; cows simply return to estrus or are found nonpregnant at a subsequent examination.[33] Taking these issues into account, normal abortion rates appear to be about 2% to 5% when only observed abortions are considered, approximately 8% to 10% or perhaps higher if one considers both unobserved and observed abortions based on pregnancy status determined by traditional rectal palpation programs.[34] As a rule of thumb, abortion rates in excess of 10% of pregnancies confirmed at 42 days of gestation or greater should be considered increased rates. More data are needed to clarify what constitutes "normal" loss of embryos prior to 42 days of gestation. Computer record programs must grapple with these definitions. Because the definition of an abortion is a pregnancy that is later determined to be open, then early pregnancy diagnosis will result in what appears to be a sharp increase in abortion rates in a herd where no biologic change has occurred. One approach is this issue is to accept a pregnancy as "confirmed" only if diagnosed after day 42. Earlier pregnancies, if lost by day 42, would fall back into the category of nonconception (or maybe as early embryonic death), rather than abortion. This compromise serves to reserve the term "abortion" for the fetal period of gestation, but combines two sources of reproductive failure (true conception failure and early embryonic death) together in one category. As diagnostic abilities increase, it seems very likely that what were once considered simple failures of conception are in large part really loss of early embryos.

Determining what constitutes a significant clustering of abortion remains problematic. In practice, waiting for statistical proof of an outbreak may not be prudent; practical importance may be established and action probably warranted well before statistical significance is reached. It is increasingly clear, however, that pregnancy diagnoses made in the first 2 months of gestation need to be reconfirmed so that lost pregnancies are identified in a timely manner and open cows are dealt with appropriately. Diagnosing the cause of abortion continues to be a challenge. There is a bias toward submitting fetuses aborted in later stages of gestation rather than younger ones, which may lead to inaccurate assessment of the major causes of fetal losses on dairies.[35] Fetal losses in earlier stages of gestation are far less likely to be observed, despite the fact that cows are more likely to lose their pregnancy in these early stages (particularly up to about day 60).[33] Serologic status of cows that abort may be similarly misleading, unless seroconversion can be demonstrated.[36]

Culling

As a reproductive monitoring tool, culling has very significant lag and momentum. By the time a cow is culled, the fundamental management problem that led to the cull (e.g., poor transition cow management, poor estrus detection) is probably distant history. Another difficulty lies in the definition of the reason an animal was culled.[37] If a low-producing cow with poor feet and legs and one blind quarter is open at 150 days post partum and the dairy manager decides to stop breeding her, is she a reproductive cull (open when culled at the end of lactation)? Thus "reproductive culling" is a very poor monitor of a herd's reproductive program. The tools described here remove all justification for ever attempting to use "reproductive culling rate" as a monitor of the reproductive program on a dairy.

MANAGING REPRODUCTION ON A DAY-TO-DAY BASIS

Monitoring for status and trends provides valuable insight into the herd's current and historical situation, but it often stops short of answering the question of what needs to be done on the dairy. As noted earlier, at any given time most cows on a dairy are not eligible for management intervention for reproduction. Record systems need to reach past the general herd status and target those cows for which action can be taken. What is often needed is not a measurement of the performance of those animals whose outcome (positive or negative) has already been resolved, but rather the identity and status of those animals for which positive management action is possible. Such cows might be past the VWP and not bred, bred but not confirmed pregnant, and so on. Given that estrus detection is the major opportunity area in the reproductive program on most dairy farms, much of the focus should be on cows that have not been detected in estrus and bred. Effective record systems allow these cows to be identified easily, often leading to efforts to induce a fertile estrus.

Data from dairy management software or downloaded DHIA records afford a significant opportunity for veterinarians to participate in this sort of "action list management" in their clients' reproductive programs. Care is necessary to be sure that lists correctly isolate those cows that are eligible for a particular intervention and that no cows are missed or incorrectly included. Many systems fail because of fundamental errors of management or implementation. It cannot be assumed on all dairies that cows have unique identification or that data are correctly entered. These basics are necessary before sophisticated programs can be implemented.

CONCLUSION

Reproduction has a central role in the management of a dairy farm. Veterinarians need to be fully involved in the reproductive management of their client herds. To do so most effectively, veterinarians must learn to routinely assess the herd's status and trends and identify both individual cows and general areas of management where intervention is possible and the client's welfare served.

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CHAPTER 62

Reproductive Health Programs for Beef Herds: Analysis of Records for Assessment of Reproductive Performance

TERRY J. ENGELKEN, CHE TREJO, and KYLE VOSS

Those operations that emphasize decreased cost of production and the optimization of production efficiency should remain competitive in the years to come. However, certain external factors outside the "farm gate" will continue to have a dramatic effect on how cow and calf producers conduct their business. The continued consolidation in the packer and retail segments, the move from commodity beef production toward branded retail products, and the globalization of world markets will certainly help shape the face of the beef industry. The need for producers to develop record systems that can track individual animals from conception to consumption will be driven by consumer demands for a safe and wholesome product, legislation that mandates country of origin labeling (COOL), and the threat of foreign animal disease outbreaks. It is a certainty that an identification system will become federally mandated in the United States in the near future.

Recent survey data in Mississippi would indicate that over 50% of cow/calf producers do not use individual animal records to make management decisions.[1] This same survey suggests that nearly 40% of these producers do not use financial records. Larger operations were more likely to use the information provided by records (both animal and financial) when making decisions compared to smaller operations. However, even in the largest herds (over 500 head), nearly one third did not use animal records in the decision-making process. Although these findings may be surprising, they represent a real opportunity for practitioners to increase their involvement in the overall reproductive management of these operations.

Most indices currently used to evaluate herd performance are directly tied to reproduction efficiency. Measures such as pregnancy rate, calving percentage, calving distribution, and percent calf crop all affect pounds of calf produced per exposed female. If reproductive performance falls below targeted levels, the practitioner is often asked to provide a plan to improve efficiency. This process requires that the producer provide or the practitioner construct adequate herd records to facilitate the evaluation process. This process will ensure that needed changes in herd management can be identified and that cost-effective solutions can be documented and evaluated over time.

DATA COLLECTION

The usefulness of available herd information is totally dependent on its accuracy and its relationship to meaningful reproductive performance measures. Data not meeting these criteria are irrelevant and only serve to make the veterinarian's task more difficult. In those situations in which records are incomplete or inaccurate, the practitioner would be better off taking time to design a system of data collection to fit the operation. This system could be as simple as an index card for each cow. It could also involve a customized spreadsheet or database application or utilize one of the currently available software packages on a laptop or PDA. Regardless of the collection system used, data are only useful if they facilitate decision making.

The minimum data required to begin any analysis[2] include factors such as cow identification, age, breed, body condition score, pasture location, and estimated days pregnant, all of which can be captured chuteside as each group of breeding females is processed. Female weights and frame score are also useful parameters to track if those numbers can be conveniently collected.

Pregnancy evaluation should be done as soon as possible after the end of the breeding season. This allows a more accurate dating of the pregnancies and an earlier indication of reproductive performance. Performance of individual breeding groups as well as the whole herd can then be compared to established targets for operations in a particular geographic region.[3] This type of reproductive information is critical in evaluating the success of the breeding program.

The bull battery represents the other half of the reproductive equation. Information concerning the day the bulls entered and left the pasture, bull breed and identification, and pasture assignment should also be collected.[3] Purchased bulls that have been added to the herd since the last breeding season should have their breeding soundness examination forms, registration papers with EPD values, and health certificates examined by the prac-

titioner. The producer should retain these records at least through the following calving season and preferably for as long as the bull remains in the herd. This information can be very useful when trying to track down the cause of reproductive losses or increased calf morbidity.

Detailed herd surveys can also be used to build an initial database.[4] Herd surveys not only give the veterinarian an overview of the operation, but also require the producer to take time and review the production practices currently in use. These surveys come in many formats; they should cover areas such as the animal health program, feeding practices, supplemental feeding, land allocation, grazing and fertilization practices, genetic selection, and physical facilities. Financial information should also be considered. Once accurate information is obtained, the veterinary clinic can act as a central data procession point to compile and analyze individual cow data and overall herd performance.

RECORD ANALYSIS

When evaluating the success of the breeding program, the traditional measure that has been most often used is percent pregnant. Taking the number of animals pregnant in the individual group or herd and dividing it by the number of exposed females will calculate this measure. Although this measure has some value, it gives no insight into important factors such as the length of the breeding season, bull-to-female ratios, grazing conditions, or cow characteristics (age, body condition score [BCS], or frame score) that have an impact on reproductive performance. A more complete analysis of herd performance would enable the veterinarian to judge when individual cows become pregnant and the percentage by each estrous cycle. A complete evaluation allows the practitioner and client to track cow and bull reproductive performance, replacement heifer development, and the herd's nutritional status.[2,3,5,6] This type of analysis leads to a better understanding of production problems, intervention strategies, and lines of communication between the producer and practitioner.

If accurate production records already exist, variations in female reproductive performance can be assessed by cow age, breed, frame score, and body condition score. However, in many cases the first opportunity to access this information occurs at the time of pregnancy examination. Although this evaluation should determine pregnancy status first and foremost, the analysis should also consider the costs involved in getting cows pregnant and the value of the weaned calf. Cost centers such as the nutritional program, heifer replacement, and grazing costs can then be related to overall profitability. Also, the timing of herd management events should be evaluated and clearly defined.[3]

Once all data are collected, this information can be summarized in tabular form as a starting point for the herd analysis (Table 62-1). As this is done, individual group and herd factors that affect reproductive performance will begin to emerge. The fall-calving herd profiled in Table 62-1 shows excellent reproductive performance across all age groups. With the exception of the expected decrease in performance of the oldest set of cows, there was no real difference in percent pregnant, average days pregnant, or body condition score. Typically, the 3-year-old females are the most difficult group to get rebred because they are nursing their first calf. In our experience, it is not unusual to see the pregnancy rate in this group of females drop 5% to 10% below the herd average, while taking 10 to 14 days longer to conceive. Because of the capital invested in the replacement heifer program, it is critical that these young females be monitored and managed to ensure acceptable reproductive performance through their first two breeding seasons.

Nutritional management can have a dramatic impact on reproductive performance.[7] Also, the supplemental feeding program typically represents the largest cash cost associated with the cow/calf operation. The nutritional status of the herd can be monitored by using a body condition scoring system to evaluate the success of the feeding program.[2] By dividing the herd into groups based on body condition, the practitioner can evaluate reproductive performance and communicate those findings to the producer. Table 62-2 details the relationship for another fall-calving herd. This particular operation was successful in managing the forage base and supplemental feeding program so that less than 10% of the herd was in borderline condition (BCS = 4) at the time of pregnancy examination. Because of the expense associated with feeding the cow herd and the critical role that nutrition plays in productivity, it is critical that this relationship be evaluated on an annual basis.

Calving histograms can be used to further define the conception pattern of the herd and the expected calving distribution.[5,6] The histogram shows the percentage of

TABLE **62-1**

Relationship of Pregnancy Status to Female Age and Body Condition Score (BCS) for a Fall-Calving Herd

Female Age (yr)	Total Number	Number Pregnant	Percent Pregnant	Average Days Preg	BCS Pregnant	BCS Open
Heifers	44	39	88.6	149	6.8	6.4
3	48	45	93.8	142	5.9	6.0
4–8	147	139	94.6	139	5.7	5.6
>9	26	21	80.8	137	5.6	5.2
Total	265	244	92.1	141	5.9	5.8

TABLE **62-2**

Mature Cow Reproductive Performance Based on Body Condition Score (BCS) in a Fall-Calving Herd

Number of BCS	Number Cows	Number Pregnant	Percent Open	Pregnant	Average Days Pregnant
4	23	19	4	82.6	153.2
5	114	107	7	93.9	149.1
6	89	81	8	91.0	144.3
7 & 8	20	20	0	100.0	142.1
Totals	246	227	19	92.3	147.1

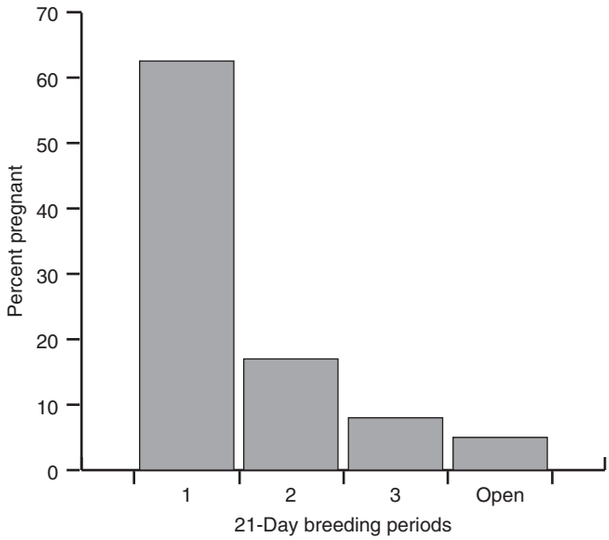

Fig. 62-1 Breeding histogram showing the percentage pregnant by 21-day periods.

females bred in each 21-day period (Fig. 62-1). This process should be done for the entire herd and then broken down into specific breeds, ages, body condition scores, or pasture locations, as deemed necessary by the practitioner. Reasonable targets for mature cows (4–8 years old), using a limited breeding season (45–75 days) include having 60% of the females conceive in the first 21-day period and less than 10% open. These targets will vary for different female groups and environmental conditions.

Pregnancy examination data and the calving histogram can be utilized to determine the median pregnancy date (MPD) for the herd.[2,6] If calving information is available, median calving date (MCD) can be used as well. A discussion on how to calculate these measures was included in the first edition of this text.[2] These measures can be used to manipulate the beginning of the breeding season to produce a more concentrated breeding and calving season. These parameters are particularly useful in herds with prolonged breeding seasons, poor conception rates in the first 21-day period, and a large number of late-calving cows. Once the MPD is calculated, the following breeding season can be "pushed back" as needed in order to give the females more time to gain condition between calving and breeding or to make sure that the breeding season better matches the forage base of the ranch. This should help producers avoid the economic penalties associated with high culling and replacement rates.

The major income-producing component of the cow/calf enterprise is the weaned calf. Therefore, it is imperative that factors affecting pounds of calf produced per exposed female be routinely monitored.[8] Factors such as dystocia, enteric disease, and respiratory pathogens lead to increased death loss of calves. Lower than expected suckling calf performance (growth rate) also contributes to poor weaning weights. Calf age and weaning weight are directly related, with early born calves being heavier at weaning (Table 62-3). Calf weaning weights are also affected by age of the dam; cows 4 to 8 years of age typically wean heavier calves than younger or older females (Table 62-4). A prolonged breeding and calving season not only results in younger and lighter calves being weaned, but also makes postweaning management of the replacement heifers more difficult. The age distribution of the females should also be evaluated over time if weaning weights are not meeting expectations.

With an annual replacement rate of 10% to 20%, the sale of cull animals represents another significant source of income for producers.[9] Besides serving as a source of income, these cows can also be used to judge how well the female selection criteria match the ranch environment. Culling rate, cow age, reason for culling, and sale price need to be monitored over time (Table 62-5). Physical abnormalities, such as ocular neoplasia and lameness, need to be addressed as part of the producer's beef quality assurance program. Disease monitoring programs can also be instituted in culled cows to evaluate the incidence of reproductive disease and the effectiveness of the herd health program.[10]

An evaluation of the financial health of the cow/calf enterprise enables the producer to quantify cost of production and owner equity status. This also gives the practitioner an opportunity to show the client how veterinary input has affected herd profitability. Information concerning only the costs and returns of the cow herd is considered.[11] Fixed inputs being used in more than one ranch enterprise (such as machinery and buildings) are charged to the cow herd based on the percentage of their use. Pounds of calf produced per exposed female is a good indicator of overall productivity. The accuracy of this type

TABLE **62-3**

Relationship of Calving Distribution to Calf Performance in a Mississippi Cow/Calf Operation

Calving Period	Number of Calves (%)	Average Wean Weight (lb)	Average Wean Age (days)	Average Daily Gain (lb)
I	56 (26)	527.6	252	1.80
II	93 (40)	513.5	231	1.90
III	47 (20)	498.1	210	2.01
IV	21 (9)	465.6	189	2.07
V	11 (5)	379.9	168	1.80
Totals	228 (100)	502.9	225	1.91

Adapted from Engelken TJ, Lehman FD, Little RD, et al: Helping beef producers improve cow culling practices. *Vet Med* 1993;88(11):1104.

TABLE **62-4**

Relationship of Female Age with Calf Weaning Weight for a Mississippi Cow/Calf Operation (1992 vs. 1999)

Cow Age (Years)	AVERAGE WEANING WT. (LB) *1992*	*1999*
2	469	506
3	445	473
4	519	501
5	499	517
6	463	530
7	459	551
8	468	532
9	432	527
10	435	533
>11	428	531
Herd average	461.7	520.1

TABLE **62-5**

Reason for Culling of Beef Cows in a Mississippi Cow/Calf Operation (792 Culls, 1991–2001)

Reason Culled	% of Total
Reproductive	60.2
Poor production	13.0
Age/dentition	4.8
Cow death loss	4.8
Musculoskeletal	4.5
Abortion/failure to calve	4.0
Calf death	3.7
Unknown	4.9

TABLE **62-6**

Selected SPA Production Measures for a Mississippi Cow/Calf Operation (1998–2001)

Measurement	1998	2001
Pregnancy percentage	95.1%	95.1%
Calving percentage	95.1%	95.1%
Calves born during first 63 days	76.9	95.1
Calf death loss	4.3%	3.3%
Weaning percentage	91.5%	91.8%
Average weaning weight. (lb)	418	583
Pounds weaned/exposed female	382	536
Raised feed acres/female	0.7	1.0
Grazing acres/female	3.6	4.0
Pounds weaned/acre	89	132

of analysis depends on the investigator's ability to select and monitor consistent input and output parameters.

If the producer is currently using an accountant to generate tax information, it should be possible to obtain the needed financial statements for an enterprise analysis. A beginning and ending balance sheet, income statement, depreciation schedule, and tax return for the fiscal year are needed. The fiscal year is determined according to when the calves are weaned. Production efficiency and financial return should be evaluated to facilitate the comparison of an operation's performance over the years and with other operations in the veterinarian's practice area (Tables 62-6 and 62-7).

INTERPRETATION

Once the needed information is available and the production and financial goals of the operation are established, the veterinarian begins the task of trend recognition, identification of inefficiencies, and instituting needed management changes.[2,3,11] Trends associated with reproductive efficiency, calf performance, and production costs must be identified. Because the effect of a disease process may actually mimic managerial or environmental effects, identifying the cause of these trends may be difficult. The practitioner, in conjunction with a diagnostic laboratory, is best suited to evaluate potential herd conditions to form a logical diagnostic plan of action. This ensures that the proper diagnostic samples are collected and the needed resource people contracted.

Management recommendations are then instituted to correct the situation.

In herds with established herd health programs, a marked decrease in total pregnancy rate or a shift toward increasing late-period breeding percentages from the previous year indicates reproductive management problems.[5,10,11] This may be a reflection of reduced bull fertility, inadequate group nutrition, the introduction of a disease-causing organism, or any combination of these. Nonpregnant cows serve as a potential resource for providing clues as to the reason for the reduced reproductive performance.[10] Reproductive tracts should be carefully palpated and diagnostic samples collected to rule out a disease process. Cervical mucus samples and uterine biopsies can be submitted for culture and histopathologic examination. The development of new diagnostic technologies, such as polymerase chain reaction (DNA probes), holds great promise in enabling the practitioner to identify infectious agents with much greater accuracy. If needed, culled cows can be followed to slaughter and their reproductive tracts examined post mortem. When this type of diagnostic work-up is combined with serologic testing, bull evaluation, body condition scoring, and nutritional evaluation, the cause of the problem is much more likely to be identified.

In herds not on a routine reproductive management program, poor reproductive efficiency often accompanies a prolonged breeding season.[2,3,5,6,11] In an evaluation of these herds, emphasis should be placed on those factors that influence the first 21-day breeding period (Fig. 62-2). The number of females cycling at the beginning of the breeding season has the greatest impact on early conception rate. This number may vary according to cow age, postpartum interval, and body condition score. By comparing the number of females found pregnant with the calving percentage, the practitioner can determine the timing of reproductive losses. This allows for the targeting of specific pathogenic organisms that are most commonly associated with pregnancy wastage in the early, middle, or late gestation.[10] Once the cause is determined, specific recommendations can be made for changes in bull battery management, herd vaccination protocols, or the herd's nutritional management.

The pregnancy distribution illustrated in Figure 62-2 could result from a disease process affecting conception or early gestation. However, it is more likely the result of having the cow herd enter the breeding season in poor body condition. This lengthens the postpartum interval and delays the onset of the estrous cycle. Then as the grazing conditions improve and the females increase body condition (periods 3 and 4), cycling resumes, and conception occurs. To prevent this pattern from recurring in subsequent years, the producer must decide if it is more economical to deliver additional supplemental feed or delay the start of the following breeding season. If the breeding season is moved back, it should be based on the median pregnancy date in this herd. The practitioner should also perform a detailed review of the operation's heifer development program. A long-term objective may require the producer to change the genetic base of the cow herd to better fit the ranch environment.

TABLE 62-7

Selected SPA Financial Measures for a Mississippi Cow/Calf Operation (1998–2001)

	AMOUNT	
Financial Parameter	***1998***	***2001***
Raised/purchased feed cost ($/cow)	$213.40	$123.34
Pre-tax ranch expense/cow	$362.40	$282.23
Unit cost of production (breakeven/cwt)	$106.79	$73.04
Net income/cow	–$125.16	$73.33
Percent equity	66.2 %	90.7 %
Percent return on assets	–2.77%	7.01%

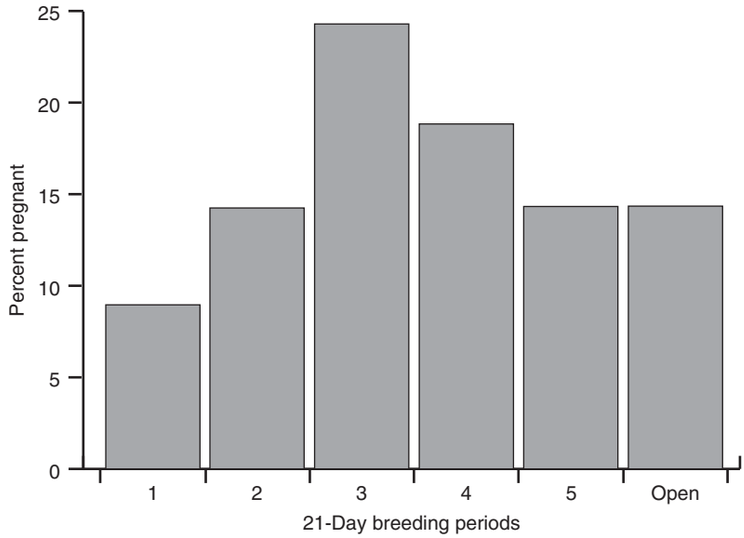

Fig. 62-2 Breeding histogram depicting a prolonged breeding season, poor conception rate per 21-day period, and a large number of open cows.

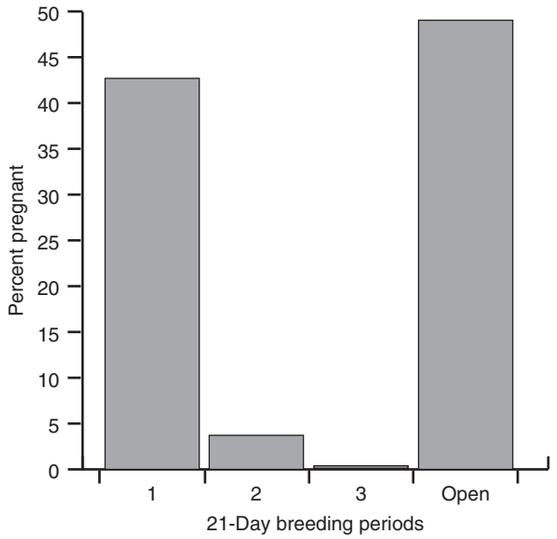

Fig. 62-3 Pregnancy distribution in a spring-calving herd following multiple bull failure.

The pregnancy distribution depicted in Figure 62-3 is not uncommon in smaller operations. The breeding season began well, but conception ended abruptly. This type of distribution most commonly occurs in breeding groups where the only bull in the pasture has been injured. The injury may be musculoskeletal in origin, but in our experience, it is most likely a preputial laceration or a penile hematoma. Often times the breeding season is lost by the time the injury is detected and a new bull turned out. This type of distribution may also occur with unseasonably hot temperatures that coincide with the spring breeding season in the southeastern United States. This effect is amplified in those situations that rely on fungus-infected fescue pastures as the dominant forage. This combination of prolonged, high ambient temperatures and increased body temperature associated with fescue intake tends to decrease semen quality and breeding activity in bulls. Producers will often comment that the bulls are spending more time under the shade or standing in ponds than "working" the breeding herd.

Mismatches between the cow and her environment can negatively affect calf performance as well. Prolonged postpartum intervals lead to younger and lighter calves at weaning. Females calving in poor body condition experience more dystocia and produce less milk. The prolonged breeding season depicted in Figure 62-2 also complicates the postweaning management of both cows and calves.[6] Owing to the wide variation in date of conception, supplemental feed budgeting for the pregnant cows is difficult. The calves are extremely uneven in size and body weight, which decreases their total value. There is also a wide range in heifer weights, making replacement selection and management more difficult. By relating the calving histogram and median calving date to a producer's production practices, the practitioner is in a position to recommend cost-effective management changes and to document their impact on ranch profitability.

Using records to periodically review herd performance is a critical component of the production medicine program. This review should focus on those management issues that were originally identified as causing production or financial inefficiency for the operation. This process may deal with the reproductive performance of the entire herd or one of the specific components (mature cows, replacement heifers, young cows, bull management, etc.) that has caused problems in the past. Key production and financial parameters should also undergo periodic evaluation to make sure that progress is being maintained.

The data in Table 62-4 represents calf weaning weight as a function of cow age. In the initial year (1992), it was noted that weaning weights were generally lower than expected and the 3-year old cows were especially affected. It was felt that the decrease in production in the young cows was due to an increase in their postpartum interval following first calving. Another concern was that the majority of the mature cows (ages 6 years and older) were weaning calves that were no heavier than those from the 2-year-old females raising their first calf.

In this situation, factors affecting weaning weight were evaluated and changes in the supplemental feeding program, replacement heifer development, and the genetic base of the cow herd were employed. Over the following 7 years, there was an overall increase in the herd's weaning weight and better production from the mature cows. However, it appears that work still needs to be done in the postpartum management of the replacement heifers to ensure timely conception during the second breeding season. This will ensure adequate production as 3-year olds. This periodic analysis of historical ranch data enables the practitioner to evaluate the success of the management program and justify its cost to the producer.

SUMMARY

The reproductive performance of the breeding herd is critical to the operation's ability to produce income. Income generation is affected not only by the absolute number of females that conceive, but also by the timing and distribution of the pregnancies. The success or failure of the breeding season can be affected by a wide range of disease and management factors. Complete and accurate record systems offer the practitioner an opportunity to recognize trends, identify problems, and implement cost-effective management changes. These record systems also document the effect of these management changes on subsequent productivity. The use of data collection and expert analysis as a means of tracking herd efficiency will become very important in these operations. Veterinarians have a unique opportunity to position themselves to take a leading role in providing cow/calf producers with this type of service.

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CHAPTER 63

Assisted Reproductive Technologies in Cattle

PETER W. FARIN, KAREN MOORE, and MAARTEN DROST

Assisted reproductive technologies (ART) have made tremendous advances, especially during the past 15 years. Artificial insemination (AI) remains the most effective method for achieving genetic progress in populations of cattle. The global market remains strong for frozen semen and embryos. Millions of cattle are bred by AI, and more than a half million embryos are transferred annually world wide. Most of the top dairy sires used for AI were derived from embryo transfer (ET). Improvements in methods of controlling the estrous cycle and ovulation have resulted in more effective programs for AI, superovulation of donor cows, and the management of ET recipients. The recent introductions of in vitro embryo production, cloning, and sexed semen have added to the ART "toolbox."

In this chapter we discuss current reproductive technologies in cattle, with particular emphasis on procedures used in the practice of ET. Our goal is to provide the reader with information and guidelines for applying ET and related technologies. The newer technologies of in vitro embryo production and cloning are also addressed. The chapter concludes with a discussion of the use of sexed sperm and embryos to preselect calf gender.

ARTIFICIAL INSEMINATION

Artificial insemination was the first assisted reproductive technology to be applied commercially for the genetic improvement of animals in the mid-1900s. The advantages of AI in terms of disease control, the import and export of frozen semen, the availability of accurate breeding records, and the elimination of dangerous bulls on farms are well established. AI has become the foundation for expanded breeding schemes such as estrus synchronization programs (synchronized breeding, including timed AI), embryo transfer, in vitro embryo production (IVP), the use of sexed semen, cloning, and transgenics. The use of AI, especially in dairy cattle, has become so routine that most of it is practiced by the producers or herd managers themselves. The main disadvantage is that the latter do not always develop sufficient skills to maintain acceptable conception rates in their herds.

EMBRYO TRANSFER

The next major commercial advancement in reproductive biotechnology was embryo transfer in the late 1970s. ET

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EMBRYO TRANSFER

The next major commercial advancement in reproductive biotechnology was embryo transfer in the late 1970s. ET

enabled the acceleration of the proliferation of genetic material from the dam as well as of the sire. The ability to freeze and transport bovine embryos around the world has made ET an extremely useful technology for disease control and biosecurity programs, genetic salvage of valuable individuals, and development of new lines or breeds of cattle. ET is a factorial process that depends on a series of carefully orchestrated sequential steps. Poor performance in any of the steps directly affects the success rate and the final outcome, the number of calves weaned.

Donor Selection

Selection of the donor is based on two major criteria: (1) genetic merit, generally determined by the owner and based on performance, and (2) reproductive soundness, as assessed by the veterinarian. The donor must be in good body condition and preferably gaining weight. She should be free from underlying diseases, a minimum of 50 to 60 days post partum, and cycling regularly. Generally, cows with a history of reproductive problems do not make good donor animals.

Donors are further evaluated by examination of the cervix, uterus, and ovaries per rectum to determine that they are free from adhesions or other palpable lesions. It is prudent to test the patency of the cervical canal with a cervical dilator for sufficient internal diameter to permit passage of a collection catheter, especially if the prospective donor is a heifer or of *Bos indicus* breeding. This prevents the occasional frustration of being unable to negotiate the cervix after a series of costly hormonal injections.

Vaccinations should be current for local diseases. Blood typing of both donor and sire prior to or at the time of embryo transfer for subsequent identification of offspring is highly recommended and is generally required prior to export.

Donor Management

Single embryos or multiple embryos may be collected from naturally ovulating or superovulated cows, respectively. For optimal efficiency 2 to 4 donors should be treated and synchronized with their recipients for each attempt; this allows sharing of the recommended potential 8 to 10 recipients per donor.

Superovulation remains the least predictable step in the process of embryo production. In the bovine tremendous variation in response occurs with age, breed, lactational status, nutritional status, season, and stage of the cycle at which treatment is initiated. Follicle stimulating hormone (FSH), which has a short half-life, necessitates twice-daily injections over a period of 4 to 5 days. Treatment is begun during the mid-luteal phase (day 8 to 12) of the donor's cycle and employs the use of prostaglandins (PG) to synchronize the cycles of the donors and the recipients. Alternatively, treatment may be initiated on day 16 or 17 (day 0 = estrus) of the donor's natural estrous cycle. Currently the most commonly used source of FSH in the United States is Folltropin-V.*

*Bioniche Animal Health USA, Inc., Athens, GA.

TABLE **63-1**

Superovulation Treatments with Follicle Stimulating Hormone in the Bovine*

Treatment Day		Treatment 1	Treatment 2
–27		25 mg (5.0 cc) PGF2α†	25 mg (5.0 cc) PGF2α
–17		25 mg (5.0 cc) PGF2α	25 mg (5.0 cc) PGF2α
–14		Estrus	Estrus
–4	AM	80 mg (4.0 cc) FSH‡	50 mg (2.5 cc) FSH
	PM	80 mg (4.0 cc) FSH	50 mg (2.5 cc) FSH
–3	AM	60 mg (3.0 cc) FSH	50 mg (2.5 cc) FSH
	PM	60 mg (3.0 cc) FSH	50 mg (2.5 cc) FSH
–2	AM	40 mg (2.0 cc) FSH	50 mg (2.5 cc) FSH
		35 mg (7.0 cc) PGF2α	25 mg (5.0 cc) PGF2α
	PM	40 mg (2.0 cc) FSH	50 mg (2.5 cc) FSH
		25 mg (5.0 cc) PGF2α	25 mg (5.0 cc) PGF2α
–1	AM	20 mg (1.0 cc) FSH	50 mg (2.5 cc) FSH
	PM§	20 mg (1.0 cc) FSH	50 mg (2.5 cc) FSH
0		Estrus and AI§	Estrus and AI§
7		Embryo recovery, transfer, and freezing	Embryo recovery, transfer, and freezing

*Treatment 1 (decreasing dosage of FSH) as preferred by most ET practitioners, Treatment 2 (level dosage of FSH) as recommended by the manufacturer.
†PGF2α, Prostaglandin F2α; Lutalyse, Pfizer Animal Health, New York; IM injections.
‡FSH, Follicle stimulating hormone; Folltropin-V, Bioniche Animal Health USA, Inc., Athens, GA; IM injections.
§FSH treatment is discontinued when the donor comes into estrus early. If the donor does not come into estrus, FSH treatment may be continued 1 additional day at the dosage level of the last scheduled day. Artificial insemination (AI) of donor at 4 to 6 hours after onset of estrus, repeated once 10 to 12 hours later.

Twice daily intramuscular injections of FSH are recommended (Table 63-1). Prostaglandins (25–35 mg $PGF_{2\alpha}$ or 500 µg PG-analogue IM) are routinely given at the time of the fifth and sixth FSH injections of a 4-day regimen, which is then followed by estrus in 2 days and ovulation in 3 days. This interval from PG to the onset of estrus is 12 to 24 hours shorter in superovulated animals than in naturally ovulating cows or heifers. Consequently, recipients should be injected with PG 24 hours before the donors if this method of synchronization is used. The response to the FSH regimen ranges from zero to 20 or more ovulations with an average of 8 to 10. There appears to be no difference in response between a 4-day and a 5-day regimen. Generally, heifers require a smaller total dose and older animals a higher total dose.

Many embryo transfer practitioners use exogenous progesterone or intravaginal controlled drug (progesterone) release devices (controlled internal drug release, CIDR)† as part of the superovulation protocol. This approach may be used simply to synchronize a group of donors in order to start the FSH treatment approximately 10 days after their synchronized heat. Alternatively, the

†InterAg Company, Hamilton, NZ; Pfizer Animal Health, New York.

TABLE 63-2

Superovulation Treatment with Follicle Stimulating Hormone and CIDR without Regard to the Day of the Donor's Estrous Cycle

Treatment Day		Treatment
0		CIDR* inserted vaginally
2	PM	100 μg GnRH†
4	PM	60 mg (3.0 cc) FSH‡
5	AM	60 mg (3.0 cc) FSH
	PM	60 mg (3.0 cc) FSH
6	AM	50 mg (2.5 cc) FSH
	PM	50 mg (2.5 cc) FSH
7	AM	40 mg (2.0 cc) FSH
	PM	40 mg (2.0 cc) FSH, 35 mg (7.0 cc) PGF2α§
8	AM	40 mg (2.0 cc) FSH, 25 mg (5.0 cc) PGF2α CIDR out
9	AM	Estrus and AI
	PM	Estrus and AI
16		Embryo recovery, transfer, and freezing

*CIDR, Controlled internal drug release; EAZI-BREED CIDR progesterone insert, InterAg Company, Hamilton, NZ, or Pfizer Animal Health, New York.
†GnRH, Gonadotropin releasing hormone; Cystorelin (Gonadorelin), Merial Limited, Iselin, NJ; IM injection.
‡FSH, Follicle stimulating hormone; Folltropin-V, Bioniche Animal Health USA, Inc., Athens, GA; IM injections.
§PGF2α, Prostaglandin F2α; Lutalyse, Pfizer Animal Health, New York; IM injections.

donors may be superovulated with the CIDR in place according to the treatment schedule in Table 63-2. The advantage of the latter approach is that the donors may be implanted at any time during their cycle. However, it is critical that the donor is a reproductively normal, cycling animal.

It is difficult to accurately assess the number of ovulations by palpation of ovarian structures per rectum on the day of embryo recovery when the number of corpora lutea (CL) exceeds 4 to 6 per ovary or when several anovulatory follicles are also present. An excessive number of anovulatory follicles in the presence of corpora lutea appears to adversely influence the percentage of recovered embryos because of an unfavorable estrogen-to-progesterone ratio, which affects gamete and embryo transport through the tubular reproductive tract.

Ultrasonography

Donor management can be enhanced by the use of real-time ultrasonography. In addition to accurate assessment of the normalcy of the reproductive tract, including ovarian status, the presence of a dominant follicle (DF) can be ascertained. The presence of an active DF can suppress ovulation rate by as much as 40%. Ablation of the DF prior to FSH treatment allows donors to be scheduled based primarily by the calendar rather than their follicular wave patterns.

Ultrasonography is helpful in assessing the potential superovulatory response on the day prior to ovulation or at the time of AI. When only one or a few follicles are observed a back-up sire can be selected rather than semen from an extremely expensive bull.

Ultrasonography and palpation of the ovaries per rectum have been shown to have similar accuracy for determination of the number of CL at the time of embryo recovery. However, the number of anovulatory follicles can be counted more accurately by ultrasonography, and this information may aid in explaining a poor response to the owner.

Estrus Detection and Insemination

Accurate estrus detection is of great importance not only for timely insemination of the donor, but also for the determination of the degree of synchronization of estrus and ovulation between the donor and her recipients. The age of the embryo is calculated from the time of onset of standing heat.

Donors should be artificially inseminated twice with a 10- to 12-hour interval, beginning 4 to 6 hours after the onset of estrus, to cover the range of time over which the ovulations may occur. Depending on the quality of the frozen semen, a double inseminating dose may be used at each insemination. A double inseminating dose should be used in cows with a large pendulous uterus.

Embryo Recovery

Bovine embryos descend into the uterus around day 4.5 (estrus = day 0) and shed their zona pellucida ("hatch") between days 8 and 10. Consequently, most nonsurgical recoveries are made between days 6 and 8.

A two-way round tip balloon catheter (French size 16 to 24) with a 30 ml inflatable balloon is used. The two-way catheter has one channel for inflation of the balloon plus a single channel for alternate inflow and outflow of flushing medium. A sterile stylet (such as the plunger of an insemination gun) is inserted the full length of the device to render it sufficiently rigid to allow introduction into the uterus under guidance per rectum.

The donor is restrained in a chute or in stocks. Nervous animals may be given 5 to 10 mg of acepromazine or another suitable tranquilizer. Feces are carefully removed from the rectum to avoid aspiration of air, and an estimate is made of the number of ovulations (CL). Epidural anesthesia is administered (4 to 6 ml of 2% lidocaine hydrochloride) to prevent defecation and straining. Fractious animals may be given epidural anesthesia with a combination of xylazine (30 mg) and sterile saline or sterile water (7 ml or sufficient quantity). *Bos indicus* breeds are more sensitive to the action of xylazine and should receive 20 mg of xylazine in a sufficient quantity, usually 7 ml, of sterile saline. Inadvertent air can be removed from the rectum with a small stomach tube attached to a wet vacuum cleaner. The vulva and perineal region are thoroughly washed with plain water and blotted dry. The tail is tied out of the way. If the cervix feels small or tortuous, a cervical dilator may gently be used to expand and straighten the cervical canal. The dilator and subsequent catheters may be covered with a sanitary sleeve before they are introduced into the vagina. This protective cover is perforated just before the instrument enters the external os of the cervix. *The rigid, relatively sharp-pointed dilator should be used with extreme caution as it can readily perforate the uterine wall when it is*

forced through the tight cervical canal. The lips of the vulva are again parted and the balloon catheter, with the stylet in place, is inserted into the vagina and advanced into the lumen of the cervix. It is then manipulated into the appropriate horn until the inflatable balloon is situated at the base of the uterine horn. The balloon is slowly inflated with 15 to 20 ml of air or flushing medium in adult cows and 10 to 15 ml of air in heifers. The endometrium can easily be split by overdistention, resulting in hemorrhage and escape of the flushing solution into the mesometrium, from which it cannot be recovered.

After the catheter is in position, the stylet is removed and the catheter is connected via a Y junction by sterile tubing to a 1000 ml bottle or bag of flushing medium. The remaining arm of the Y junction is connected to a free piece of tubing. The flow of medium in both pieces of tubing is controlled by quick-release clamps. While the outlet tubing is occluded, the flushing solution enters the uterus by gravity flow with the bottle suspended approximately 1 meter above the level of the uterus. The horn of the uterus is extended by elevating the tubouterine junction and by carrying it anteriorly. When the inflow stops, the inlet tubing is clamped off and the clamp on the outlet tubing is released. The fluid is channeled directly through an embryo filter (75 µm pore size).

In older animals with long pendulous tracts, manipulation of the cervix and uterus can be facilitated by retracting the cervix into the vagina with cervical forceps. If the returning fluid is blood-tinged, the red blood cells may be washed directly through the filter by opening both clamps between the bottle of flushing solution and the filter. The filter should never be allowed to run completely dry, leaving the embryos on the filter disk exposed to the air.

In superovulated animals the procedure is repeated for the opposite horn, using a separate sterile catheter. It is hazardous to reinsert the stylet into the balloon catheter while it is in the uterus because the sharp tip might exit through one of the side openings. Some operators prefer placement of the catheter with the balloon just anterior to the internal os of the cervix, in the body of the uterus, which enables them to flush both horns simultaneously. In older animals the balloon is frequently displaced to this body location during the filling and stretching of the uterus even though the balloon was initially placed in one of the horns. When this happens, both horns are simply flushed at the same time (body flush).

Flushing and Holding Media

The most commonly used medium for nonsurgical embryo recovery is Dulbecco's phosphate buffered saline (PBS). One percent heat-treated bovine serum (10 ml) is added to each individual 1 L bottle of flushing medium, which may be used at room temperature. Serum acts as a protein source for embryo growth and membrane stabilization, and renders embryos less sticky. In lieu of serum, 0.04% bovine serum albumin (BSA) may be used for the recovery medium and 0.4% for the holding medium.

Ten to 20% serum is added to the flushing medium to make a holding medium that can also be used for short-term (less than 24 hours) culture. Holding medium should be sterilized by filtration through a 22 µm Millipore filter attached to an all-plastic syringe. The rubber plungers of some syringes have been shown to be coated by an embryotoxic lubricant; hence it is recommended that all-plastic syringes be used.

Holding dishes should be kept covered to minimize contamination and evaporation. The latter will increase the osmolarity of the medium. Changing the embryos to a fresh dish of holding medium periodically (every 2 hours) further minimizes the effects of contamination and evaporation.

By the very nature of the procedure, it is vital that all aspects of **quality control** of media and equipment that come in contact with the embryos are strictly adhered to. It is also advisable to use commercially prepared media and sterile disposable supplies.[1]

Embryo Handling and Evaluation

Identification and evaluation of embryos is one of the most challenging aspects that confronts the embryo transfer practitioner, especially the beginner. Embryo quality and poor handling techniques can directly affect pregnancy rates. A stepwise procedure for embryo searching is presented at the end of this section.

Environment

Once removed from the stable protective environment of the uterus the embryo should be handled with respect regarding its temperature, pH, osmolarity, and contaminants, factors that may affect viability. Embryos depend on the ambient fluid to maintain their physiologic integrity and as a source of nutrients.

Embryos may be maintained at room temperature for several hours without decreasing pregnancy rates significantly, provided the embryos are transferred to fresh holding medium every 2 hours. Storing embryos in a temperature-controlled portable incubator is recommended for long distance transportation. There is no obvious decrease in pregnancy rates after storage at these temperatures for 12 to 24 hours. On the other hand, embryos do not tolerate temperatures above body temperature (39°C) very well.

The physiologic range of proper pH for embryos is from 7.1 to 7.5. Thus, the pH of flushing and holding media needs to be within this range. Even a slight change in the salt concentration (osmolarity) of the medium can effectively reduce the viability of embryos. If the salt concentration of the flushing or holding media is below that of the uterine environment, embryos will absorb water and swell to reach osmotic equilibrium, which sometimes results in rupture of the cell membrane. Conversely, if the salt concentration is above that of the uterus, the embryo will shrink in size (dehydration), causing a reduction in metabolic activity. In comparing the two situations, although both are detrimental to embryos, shrinkage would be less detrimental. If PBS is prepared from a powdered mixture, care should be taken that the correct amount and quality of water is added. The normal osmolarity of uterine fluid is 270 to 300 milliosmoles.

Exposing the embryos to ultraviolet rays for a prolonged period may cause cellular death. The use of insecticide sprays in the embryology room should be avoided. Insufficient time of aeration after using ethylene oxide gas for sterilization of equipment is detrimental to live cells. Storage period, different suppliers, and batches (lot number) of sera all affect embryo growth differently.

Identification of Embryos

Evaluation of the embryo in the uterine effluent is based on identification of several morphologic features of the embryo using light microscopy. This is the only practical method to determine suitability of the embryo for transfer and freezing. These methods are subjective and depend on experience.

The embryo is **spherical** and is composed of cells (blastomeres) surrounded by a gelatin-like shell and acellular matrix known as the **zona pellucida.** The zona pellucida performs a variety of functions until the embryo hatches, and is a nice landmark for embryo identification. The zona is spherical and translucent; thus, it is clearly distinguishable from cellular debris. Because of its shape the embryo tends to roll on the bottom of the searching dish.

The overall **diameter** of the bovine embryo is 150 to 180 μm including a zona pellucida thickness of 12 to 15 μm. The diameter remains constant until expansion of the blastocele begins. The color of the morula and (early) blastocyst also facilitates identification because the embryo is usually darker than other uterine debris. Knowing the age of the embryo (days after onset of estrus) is also important in locating the embryo in the searching dish. The fully expanded blastocyst possesses a thinner zona pellucida and is pale (translucent) in color. A spontaneously hatched embryo is very hard to identify because the embryonic mass, without the zona pellucida, is morphologically similar to uterine debris. If the hatched embryo has collapsed, it may still be identifiable but with considerable difficulty. In summary, the important criteria in identifying embryos are (1) shape of the embryo, (2) presence of a zona pellucida, (3) size, (4) color, and (5) knowledge of the age of the embryo.

During embryonic development, cell numbers increase by geometric progression. Synchronous cell division is generally maintained up to the 16-cell stage in embryos. After that, cell division becomes asynchronous and finally individual cells possess their own cell cycle. These cells composing the embryos are termed blastomeres and are easily identified up to the 16-cell stage as spherical cells. After the 32-cell stage (morula stage), embryos undergo **compaction.** As a result, individual cells in the embryo are difficult to identify beyond this stage. The most obvious morphologic manifestation during compaction is the loss of a concise cellular outline. The embryo proper develops from the **inner cell mass,** whereas the surrounding **trophectoderm** primarily gives rise to the chorionic ectoderm of the placenta.

Handling the Embryos

Once an embryo is identified in the searching dish, it is immediately transferred to a small Petri dish (35 × 10 mm) containing fresh, filtered (0.22 μm pore size), sterile holding medium. Embryos are tentatively classified simply as good or bad, and may be recorded on the cover of the holding dish. This allows for a quick account of the total number of embryos found. Embryos are then serially rinsed through at least three different dishes containing fresh sterile medium using a new sterile pipet for each step. Finally, they are placed into a dish awaiting transfer or cryopreservation. Under some circumstances (e.g. for export of embryos) they *must be* rinsed through 10 different dishes containing sterile media and exposed to trypsin.[1] All dishes must be kept covered between searches to avoid contamination, and particularly evaporation, when placed in the incubator. Evaporation of the small volume of medium in a flat dish rapidly leads to hypertonic solutions.

Classification of Embryos

Embryos recovered 5 to 8 days after estrus are **classified morphologically** into the following groups, based on their stage of development. Proper evaluation requires rolling of the embryos along the bottom of the dish.

Morula: Blastomeres are round in shape and are not tightly connected to each other. Individual blastomeres are difficult to discern from one another. The cellular mass of the embryo occupies most of the perivitelline space.

Compact morula (tight morula): The shape of a tight morula is similar to a golf ball, in that the outer edge is slightly undulated (scalloped) in appearance because of compaction. Individual blastomeres are no longer distinguishable. Cells on the surface of the mass are polygonal in shape. The embryo mass occupies 60% to 70% of the perivitelline space.

Early blastocyst: A tiny transparent (clear) space that contains fluid is visible. This area is the beginning of the blastocele. The embryo occupies 70% to 80% of the perivitelline space.

Blastocyst: The prominent blastocele cavity accounts for more than 70% of the volume of the embryo. Two groups of cells are present and clearly recognizable as the trophoblastic layer beneath the zona pellucida and the darker inner cell mass occupying one side of the embryo. The perivitelline space may still be visible but is very small.

Expanding or expanded blastocyst: There is no perivitelline space between the layer of trophoblastic cells and the inside of the zona. The zona pellucida becomes thinner as the blastocyst expands. A small well compacted inner cell mass positioned on one side of the embryo is observed. The color of the embryo is pale to clear because of the large amount of fluid present inside.

Collapsed blastocyst: There is perivitelline space along with a very thin zona pellucida. The blastocyst may be partially collapsed, with a smaller blastocele cavity, or completely collapsed and having the appearance of a compact morula.

Hatched blastocyst: Ultimately the blastocyst expands to the point of rupture and the embryo escapes from the disrupted zona. Hatched blastocysts may be spherical with a well-defined blastocele or they may be collapsed, resembling debris. Identification of embryos at this stage can be difficult for the inexperienced operator.

When zona-free, or hatched, blastocysts are collected, there is a greater risk of damage due to handling. Furthermore, hatched blastocysts are sticky and may adhere to tubing and glassware. Embryo filters should not be used when there is a possibility that hatched embryos will be recovered (>day 7.5).

Embryos are then classified according to **quality** based on morphologic appearance. Excellent/good, fair, and poor quality embryos are considered transferable into recipients. Excellent or good quality embryos (Code 1) are freezable.

Codes for Embryo Quality[1]

Code 1: Excellent or good. Symmetrical and spherical embryo mass with individual blastomeres (cells) that are uniform in size, color, and density. The embryo is consistent with its expected stage of development. Irregularities should be relatively minor and at least 85% of the cellular material should be an intact, viable embryo mass. This judgment should be based on the percentage of embryo cells represented by the extruded material in the perivitelline space. The zona pellucida should be smooth and have no concave or flat surfaces that might cause the embryo to adhere to a Petri dish or a straw.

Code 2: Fair. Moderate irregularities in overall shape of the embryo mass or size, color, and density of the individual cells. At least 50% of the cellular material should be an intact, viable embryo mass.

Code 3: Poor. Major irregularities in shape of the embryo mass, or size, color, and density of individual cells. At least 25% of the cellular material should be an intact, viable embryo mass.

Code 4: Dead or degenerating. Degenerating embryos, oocytes, or 1-cell embryos are nonviable.

Loading the Straw

Immediately prior to nonsurgical transfer, embryos are loaded individually in sterile 0.25 ml French straws. The embryo is aspirated from the holding dish into the straw with the aid of a 1 ml tuberculin syringe attached to the plug end of the straw. First a 3 cm column of medium is aspirated into the straw, followed by a 0.5 cm column of air, then a 3-cm column of medium containing the embryo, followed by another air bubble. The remainder of the straw is filled with medium until the initial column of medium wets and solidifies the plug.

Recipient Selection and Synchronization

1. Recipients should be large-framed, healthy, mature young cows or heifers in good body condition. A minimum of two normal cycles should ideally have been recorded before use, whether they will be synchronized with prostaglandins or selected from a pool of cycling animals. It has been common in embryo transfer programs to overlook the quality of the recipients. Recipients and their maintenance represent the greatest single cost of running a commercial bovine embryo transfer program. Culls from a breeding program, animals in poor condition, and early postpartum animals do not make suitable recipients. Recipients should not be fat and should preferably be gaining 0.1 to 0.2 kg per day. They should be vaccinated for the common abortion diseases.
2. Synchronous recipients can be produced in three ways: (1) Selection from a large pool of cycling females. This strategy limits the number of embryos and time when embryos can be collected. Approximately five percent of the herd will be in heat on any given day. (2) Estrous cycles of any number of recipients can be synchronized with $PGF_{2\alpha}$ or its analogues, or with CIDR devices, to exhibit heat the same day as or just ahead of the donor. (3) Timed ET, analogous to timed AI (Ov-Sync), can also be used. The importance of close synchrony between the age and the stage of development of the embryo, and the endocrine status of the endometrium of the recipient must be emphasized. Pregnancy rates following embryo transfer are best when the recipient is in estrus from 36 hours before to 12 hours after the donor.
3. The average bovine donor yields 6 to 8 transferable embryos. Therefore, 8 recipients per donor is a reasonable number to prepare. When recipients are palpated for corpora lutea, about 12 need to be palpated in order to definitely identify 8 with active corpora lutea. Not all donors will respond to the superovulatory treatment or yield transferable embryos. For optimal efficiency, two to four donors should be superovulated at the same time, which permits sharing of the prepared recipients and avoids the expensive disappointment encountered too frequently when only one donor is prepared at a time.
4. It is sometimes difficult for the embryo transfer practitioner to locate and identify a CL at the time of transfer to a synchronized recipient. Ultrasonography can identify a solid CL (>12 mm) or a fluid-filled CL with at least 3 mm of luteal tissue surrounding the central cavity. Not every recipient needs to be examined by ultrasonography, but if there is a reason for doubt, the presence of a suitable CL can be confirmed with ultrasound.
5. Embryos are routinely transferred about 7 days after the onset of estrus. Nonpregnant recipients and occasional pregnant recipients will show estrus 14 days after transfer. These animals may be examined by palpation, milk progesterone determination, or ultrasound to confirm the occurrence of true heat and the absence of a CL.
6. Early identification of nonpregnant recipients allows efficient and economical re-use of suitable animals. Of cows diagnosed pregnant using ultrasonography at 28 days after AI, 13.5% experienced early embryonic death by 56 days after AI in one study. Therefore, cows diagnosed

pregnant at 28 days of gestation by ultrasound should be re-examined around 60 days of gestation. This is also a good time (55 to 70 days) to use ultrasonography for gender determination of fetuses with unique genetic merit.

Embryo Transfer Procedure

Bovine embryos are transferred directly via the cervix, analogous to artificial insemination. It is important to minimize contamination of the uterus because it is more susceptible to infection during the luteal phase. Feces are evacuated from the rectum and the ovary containing the corpus luteum is determined. Epidural anesthesia is induced to prevent straining and defecation. The perineal area is washed, and the vulva is blotted dry.

In the laboratory, the embryo is aspirated into the 0.25 ml French straw between two air pockets and two columns of culture medium as described above. The straw is inserted into the transfer syringe and shortened to fit even with the end of the gun. A sterilized sheath is fitted over the transfer gun. A second, larger plastic sleeve, which is closed at the distal end, is fitted over the first to serve as a protective cover and permits passage of the gun through the vagina without coming into contact with the vaginal flora. The tip is placed into the external os of the cervix and is then pushed through the outer plastic sleeve before it is guided through the cervical canal and as gently as possible into the uterine horn on the side of the corpus luteum. The embryo is then gently deposited approximately one third of the way into the uterine horn ipsilateral to the CL and the gun is slowly withdrawn. A negative correlation has been demonstrated between the time spent manipulating the cervix and the uterine horn, and the pregnancy rate. Trauma to the delicate endometrium causes bleeding, and blood (complement in the serum) is embryocidal. Success is related to dexterity and practice, and pregnancy rates achieved by most operators improve with experience.

Donor and Recipient Aftercare

It is common practice to treat the donor with prostaglandins after the collection of embryos. The ovaries are generally greatly enlarged by the excessive number of corpora lutea. This treatment will not only rapidly reduce the size of the ovaries, but also terminates any unwanted pregnancies should an occasional embryo not have been flushed out. It is best not to breed the donor on this induced heat 3 to 5 days later. Furthermore, like a spontaneous heat, the induced heat will have a salutary effect on uterine health should any contamination have been introduced at the time of the collection.

Cows can be superovulated at 2-month intervals for three treatments without an appreciable decrease in embryo recovery rate. Single embryo collections may be made when the animals come into estrus and are inseminated between these superovulatory treatments.

Pregnant recipients should basically be managed like other pregnant cows. Overly fat recipients are undesirable. When the recipients have reached day 21 to 24 of their cycle (14 to 17 days after transfer on day 7), a presumptive diagnosis of pregnancy can be made based on palpation of a CL, or indirectly by determining an elevated concentration of progesterone in the milk or plasma. The conceptus may also be identified by ultrasonography at about day 26 or 27. Definitive pregnancy examinations are made at 5 weeks after transfer and confirmed at 3 months by palpation per rectum.

CRYOPRESERVATION

Cryopreservation of embryos offers several important logistical and economic advantages. First, since generally two or more donors are superovulated and flushed at the same time, there may be an excess of embryos for the number of available, synchronized recipients. Second, when the number of prepared recipients exceeds the number of fresh embryos, previously frozen-thawed embryos may be used, for the cost of maintaining large numbers of nonpregnant recipients over an extended period of time is high. Third, frozen embryos can be marketed directly by the owner of the donor, eliminating the need for shipment of pregnant recipients. Fourth, importation and exportation of frozen embryos can be an important method of disease control.

The major physical and chemical consequences of cryopreservation are the removal of pure water from the solution, which results in the formation of ice crystals and an increase in the concentration of solutes. These processes are influenced by the type of freezing medium, the osmolarity and pH of the fluid, and the rate of cooling. Damage to the embryos (cells) is caused by internal ice crystal formation, the ion concentration, and the interaction between these two physical factors. Cryoprotectants are used to protect the cells during the freezing and thawing procedures by changing the size and shape of the crystals. Both penetrating cryoprotectants, which are small molecules such as glycerol or ethylene glycol, and nonpenetrating large molecules, such as sucrose, are used.

The first prerequisite for success with any of the cryopreservation methods is to select only the best quality embryos (Code 1) for freezing. Advanced morula to blastocyst stage embryos have the highest survival rate. Some of the cells comprising the embryo will be killed/damaged by freezing and thawing. Lower grade embryos usually already have a number of dead cells and hence an insufficient number of cells to survive the freezing process, which results in unsatisfactory pregnancy rates. Embryos should be frozen within 3 to 4 hours after recovery.

It is critical to follow freezing protocols carefully and with attention to detail to minimize prolonged exposure to the cryoprotectant, which is toxic to the embryo. Freezing machines must be calibrated accurately to avoid seeding at temperatures above –5° C. Seeding is the process of induction of the formation of small ice crystals by touching the straw containing the embryo with a supercooled instrument. The small ice crystals subsequently spread throughout the solution and prevent the sudden formation of large ice crystals, which might injure the cells of the embryo. Other prerequisites include proper sealing of the straws to keep liquid nitrogen from entering the straw, which results in explosion of straws

upon thawing, and again avoiding prolonged exposure to the cryoprotectant during thawing.

Slow Cool Freezing Protocols

Currently bovine embryos are frozen by slow cool freezing methods using either ethylene glycol or glycerol. Ethylene glycol has the advantage that embryos can later be thawed and transferred directly, but glycerol has to be removed in stepwise fashion upon thawing and this procedure requires the use of a microscope and time. Freezing protocols are basically the same for ethylene glycol and glycerol. Ethylene glycol is a much smaller molecule and hence penetrates more rapidly but is much more toxic to the embryo than glycerol. To freeze for **direct transfer**, embryos are equilibrated for 5 to 10 minutes in 1.5 M ethylene glycol in PBS, part of which can be done while loaded in the straw at room temperature. At this temperature (–6 to –7° C) the straw is held for an additional 5 minutes, after which it is cooled at a rate of 0.5° C per minute to –33° C. After holding for 15 minutes at –33° C the straws are plunged directly into liquid nitrogen.

When freezing with glycerol, it is simplest to use a commercially prepared liquid embryo freezing medium, which contains PBS + 0.4% BSA + 10% glycerol. To freeze, embryos are equilibrated for 10 to 15 minutes in the 10% glycerol freezing medium. The remaining steps are the same as the preceding steps for ethylene glycol (in the freezing unit at –6 to –7° C for 5 minutes, seeded, held for an additional 5 minutes, and cooled at a rate of 0.5° C per minute to –33 C).

Each embryo is aspirated into an individual 0.25 ml straw by inserting the straw, plug end first, into the tip of a tuberculin syringe. The straw should first have been rinsed with freezing medium (up to, but not including, the cotton plug) to remove any toxic residues.

The 0.25 ml straw is loaded in the following order: a column of freezing medium, a bubble of air, the embryo in a column of freezing medium, a bubble of air, a column of freezing medium, so that the straw is full when the initial column wets the cotton plug. Both ends are then sealed with a small plug.

The Manual of the International Embryo Transfer Society provides detailed guidelines for labeling straws.[1] Half-length 0.50 ml straws are labeled beforehand. Accurate identification of the embryo(s) in each straw is imperative and should include identification of the donor registration number and the semen code.

Identification of Embryos for Direct Transfer (with Ethylene Glycol)

The *Manual of the International Embryo Transfer Society* stipulates the following for all embryos frozen for *direct* transfer[1]:

1. They must be frozen in a yellow translucent straw. This means that the straw, though yellow, is sufficiently transparent so that the embryo and the air locks are clearly visible. Such straws are available from vendors.
2. The letters DT precede the straw number on the label and the vendors will preprint this on yellow translucent straws if requested. The individual straw number may drop to the second line of the label when necessary but not separate from DT that precedes it.
3. If the label is on the plug extended from the end of the straw in which the embryo is frozen or if the label is on or within an extension of the straw in which the embryo is frozen, such plug or extension must be yellow.
4. The goblet in which embryos frozen for direct transfer are stored must be yellow.
5. The head of the cane that holds a yellow goblet that holds embryos frozen for direct transfer must be yellow.
6. Yellow should not be used for any other straws, goblets, labels or cane heads.

Thawing Protocol for Embryos Frozen in Glycerol

1. Remove the straw from the liquid nitrogen and hold it in the air for 15 seconds followed by 15 seconds in 37° C water.
2. Dry the straw and recover the embryo by cutting the heat-sealed end of the straw with clean scissors or by removing the plastic plug, and expelling the embryo by using the cotton plug as a plunger.
3. Glycerol is removed from the embryo by dilution in four steps lasting 6 minutes each: (1) 6% glycerol plus 10% sucrose in PBS plus 0.4% BSA; (2) 3% glycerol plus 10% sucrose in PBS plus 0.4% BSA; (3) 10% sucrose in PBS plus 0.4% BSA; (4) PBS plus 0.4% BSA. Instead of 0.4% BSA, 10% heat-inactivated serum may be used. Thawing media may also be purchased commercially.
4. The embryos should be evaluated as soon as possible after removal of the cryoprotectant. The embryos are frequently slightly darker in appearance, and the inner cell mass is smaller than that of fresh embryos. Occasionally the zona pellucida is cracked. If procedures are done properly, less than 5% of the embryos will have degenerated. Degenerated embryos should generally be discarded; however, if recipients are available they may be transferred anyway; a few will turn into calves.
5. Embryos should be aspirated from the final dilution medium into a rinsed, new 0.25-ml straw and immediately transferred to synchronized recipients.

If procedures are carried out correctly, pregnancy rates will be about 80% of those for unfrozen embryos transferred under similar circumstances.

Protocol for Direct Transfer

1. Line up recipients: (a) verify heat date, which should be 7 days earlier in most cases; (b) palpate for the presence of a corpus luteum and mark the appropriate side of the recipient with a crayon; (c) clip/scrub/disinfect for epidural anesthesia; (d) inject 5 ml 2% lidocaine to cows, 4 ml into heifers.

2. Prepare DT straw: (a) place liquid nitrogen tank and thawing unit side by side; thawing unit temperature should be 30°C (90°F) or less; (b) identify cane and straw; (c) remove straw, hold in air for 5 seconds, then place in thawing unit for 20 seconds while swirling; (d) dry straw, cut straw, and load straw in transfer syringe; (e) cover syringe with sanitary sheath.
3. Transfer immediately by placing the embryo one third of the way into the horn ipsilateral to the CL.

Vitrification Freezing Protocols

Vitrification is a newer alternative for freezing embryos that uses a higher concentration of cryoprotectants, avoids ice crystal formation, and places embryos in a glasslike state. It is very fast, requires less sophisticated equipment, and is thought to reduce the chilling and osmotic injury seen in slow cooling methods. The high toxicity produced by increased concentrations of cryoprotectants is avoided by quickly loading embryos into special straws, cryoloops, or droplets and directly plunging them into liquid nitrogen. One drawback to vitrification, however, is that direct embryo transfer results are not optimal, so embryos must first be rehydrated in a stepwise fashion before transfer.

Currently, there are several protocols for embryo vitrification. The most commonly used method[2] involves pre-equilibrating embryos in 7.5% ethylene glycol (EG): 7.5% DMSO in M199/20% FBS for 3 minutes, followed by quickly transferring embryos into vitrification medium (16.5% EG:16.5% DMSO in M199/20% FBS), loading them into open pulled straws and rapidly plunging them into liquid nitrogen. Vitrified embryos are thawed by immersing them in 0.25 M sucrose in holding medium for 1 minute and then transferring them to 0.15 M sucrose in holding medium for 5 minutes, followed by two 5-minute incubations in holding medium. Embryos can then be placed into culture or transferred to synchronized recipients, as described above. This method results in 7% to 95% viability after thawing, and pregnancy rates of 33% are common.

IN VITRO PRODUCTION OF EMBRYOS

In vitro production of embryos (IVP) refers to the use of laboratory procedures to generate embryos for transfer, freezing, and other biotechnologies including cloning and transgenesis. In vitro production systems offer additional options to conventional ET for obtaining embryos from donor cattle of superior genetic merit. The first calf resulting from the transfer of an embryo derived from an in vivo–matured oocyte, fertilized in vitro was reported by Brackett and colleagues in 1982.[3] Currently, embryos are routinely produced in laboratories world wide using a variety of embryo culture systems. In cattle, IVP typically consists of the following four components: retrieval of oocytes from ovarian follicles, in vitro maturation of oocytes (IVM), in vitro fertilization (IVF), and in vitro culture (IVC) of presumptive zygotes to the morula or blastocyst stage of development. This section will address the applications of IVP in cattle, as well as provide an overview of IVP procedures and expected results.

Applications of IVP

The development of effective methods for retrieving oocytes from donor cows and the concurrent refinement of in vitro culture systems to produce large numbers of embryos have resulted in creative applications of IVP in cattle. In vitro embryo production systems are used in the cattle industry, the biotechnology industry, the conservation of rare or endangered breeds, and research. Furthermore, IVP is an integral part of cloning, as discussed below. In the cattle industry, production of embryos in vitro is an alternative technology to conventional ET for generating embryos using sexed or unsexed sperm, and ultimately calves from the transfer of these embryos. Embryos produced in vitro may be transferred into recipients on day 7 or 8 of the estrous cycle or frozen for future transfers.

Oocyte retrieval and in vitro embryo production may be practiced in nonpregnant cows and heifers, pregnant cows up to about 110 days of pregnancy, and postpartum cows not responsive to FSH treatment for superovulation. Additional candidates for IVP include cows with a history of poor response to superovulation treatments, cows with reproductive problems such as acquired blockage of the uterine tubes, cows with terminal illness, and cows at slaughter. Large numbers of embryos may be generated using oocytes obtained from ovaries at an abattoir. In vitro embryo production may also be used to make half-sib embryos for transfer by fertilizing in vitro–matured oocytes with semen from different sires. Cattle producers will often alternate between conventional superovulation and IVP in attempt to increase embryo yield from valuable donor cows. In vitro embryo production services are commercially available at several embryo transfer companies and universities.

Oocyte Recovery and Transport

The first step in the IVP procedure is to obtain potentially competent oocytes from ovarian follicles of the donor cow. Ovarian follicles may be aspirated using laparoscopy, or more commonly by using ultrasound-guided **transvaginal oocyte retrieval** (TVOR). In cases of genetic salvage, the ovaries may be surgically removed using colpotomy in mature cows or by standard flank approach ovariectomy in heifers or cows.[4,5] Oocytes can also be harvested from ovaries obtained at an abattoir.

The skilled practitioner can learn to harvest oocytes from the ovaries of cows on the farm using ultrasound-guided TVOR. Briefly, the cow is restrained in a squeeze chute, and the external genitalia and perineal area are cleaned with a Betadine scrub, rinsed, and dried. A standard epidural block of 2% lidocaine hydrochloride is administered to the donor cow, and a sedative may also be administered as needed. The transvaginal aspiration system consists of a good quality ultrasound unit fitted with a 7.5 MHz probe, a vacuum pump and regulator, a probe handle for housing the ultrasound probe, and aspiration needle. The hub of the aspiration needle is attached by tubing to an oocyte collection container, such as a 50 ml conical centrifuge tube or an embryo

collection filter. An additional piece of tubing connects the oocyte collection container to the vacuum pump. The aspiration needle and tubing are first flushed with medium containing heparin. The probe handle containing the ultrasound probe and aspiration needle is then inserted into the vaginal vault. The operator's opposite hand stabilizes the ovary near the cranial vagina using per rectum palpation technique. As ovarian follicles are visualized on the ultrasound screen, the operator carefully advances the aspiration needle through the vaginal wall and pierces follicles to be aspirated with the needle. Follicular fluid and oocytes are aspirated into the collection tube or filter using a vacuum pressure of about 75 mmHg.

Oocyte retrieval can begin in cows at approximately 30 days post partum, a time when cows are not usually responsive to superovulation treatment. The TVOR procedure can be repeated as often as twice weekly until the desired number of oocytes is obtained from the cow. Each TVOR procedure takes approximately 30 minutes to perform. Oocyte yield following TVOR attempts is variable between donor cows; however, 4 to 6 oocytes suitable for IVP are commonly obtained from a healthy donor cow.[6,7] In a large study at a commercial ET facility, the mean number of oocytes per TVOR attempt in Holstein donor cows ranged from 1.5 to 10.9 oocytes with 7.2% of oocyte collections yielding no oocytes.[6]

Recovered oocytes in follicular fluid and medium should be maintained at 39° C. Bovine oocytes may be shipped to an IVP facility using counter-to-counter or overnight services. Oocytes may be shipped in oocyte maturation medium held in a temperature-controlled portable incubator. Ovaries for shipment should be held in physiological saline with 0.75 μg/ml penicillin in a sealed thermos bottle, and shipped in a cooler or Styrofoam box containing warm packs as needed. After arrival at the IVP facility, oocytes are aspirated from follicles and placed in oocyte maturation medium. Frozen semen to be used for IVP should be shipped to the facility either prior to or at the same time as shipment of the donor's oocytes or ovaries. Ideally, the semen should first be tested in the IVP system using oocytes obtained at a slaughterhouse.

In Vitro Procedures and Results

In the laboratory, immature oocytes with their cumulus cells (cumulus oocyte complexes, COC) are washed in modified Tyrodes medium (TL-Hepes), and matured for approximately 22 hours in vitro using tissue culture medium-199 (TCM199) with supplements. At the end of the maturation period, COC are placed in fertilization medium (TALP medium with supplements) with thawed frozen spermatozoa selected for high motility using swim-up or Percoll sperm separation procedures. Gametes are co-incubated for 18 to 20 hours after which time presumptive zygotes are washed in TL-Hepes and placed into culture medium. Bovine embryos have been successfully cultured to the blastocyst stage using undefined media (commonly, TCM199 plus co-culture cells, serum, and other components), semidefined media (e.g., modified synthetic oviductal fluid, SOF with BSA), and fully defined media (e.g., SOF with polyvinyl pyrrolidone, PVP).

The yield of transferable quality embryos after IVP varies from about 20% to 40% or greater. Embryo yield from cows in poor body condition, terminally ill, or infertile is often low and more unpredictable than that from healthy cows. The consistent production of good to excellent quality morulae and blastocysts from an IVP system requires meticulous attention to detail. A number of factors can influence the survival of embryos produced using in vitro systems including medium composition, atmosphere, oocyte quality, and embryo genotype.

Acceptable pregnancy rates can be achieved following transfer of in vitro–produced embryos; however, these pregnancy rates are often lower than those seen after transfer of in vivo–produced embryos.[6,7] Pregnancy rates of recipients following transfer of in vitro–produced embryos of grade 1 (good/excellent) were greater than those for embryos of grade 2 (46.9% versus 35.6%; 60% versus 46%). In addition to embryo quality, pregnancy rates after transfer of embryos produced in vitro are influenced by embryo culture medium, stage of embryo development, fresh versus frozen embryos, and synchrony of embryo development and recipient's day of the estrous cycle.

CLONING

Cloning is the production of a copy or copies of an individual and occurs in animals either naturally or artificially, when an embryo is split to produce identical twins. The word "clone" has also been used to describe animals produced by nuclear transfer for the production of an unlimited number of genetically identical offspring. The first successes in cloning livestock were with sheep, by fusing a cell from a 16-cell embryo to an oocyte that had its chromosomes removed (enucleated oocyte). From that time through the early 1990s several groups began developing this technology for the commercial production of cloned beef and dairy cattle. However, the biggest breakthrough in nuclear transfer came when it was demonstrated that a viable offspring could be produced by fusing cultured adult somatic cells with enucleated oocytes to produce Dolly the sheep in 1997. Cloning with adult cells offers the advantages of cloning from genetically proven animals, and production from cultured cells ensures an unlimited supply from which to clone. Somatic cell cloning has now been successful for cloning cattle, sheep, horses, mules, goats, pigs, many laboratory animals, as well as dogs and cats. This topic is covered in greater detail in our review.[8]

Applications of Cloning

The main application of cloning to the livestock industry is for expanding superior genetics. Animals of high genetic merit, male or female, can be selected for cloning based on any desirable trait, including growth, feed efficiency, and disease resistance. Production of numerous animals of exceptional genetic merit would allow for more rapid genetic progress and the associated economic benefits. The nutrition, reproduction, and health of these animals should be more easily managed because of animal uniformity.

Nuclear transplantation has also been useful for salvaging old genetics and exotic breeds. Germ plasm from a breed of cattle on Enderby Island that had essentially gone extinct was preserved using somatic cell nuclear transfer from the only remaining cow. Additionally, this technology can be used to import and propagate new or exotic breeds of cattle that may offer other desirable genetic traits not currently available for use in cross breeding schemes. Moreover, tissues can be harvested from recently deceased animals and propagated for use in cloning. This also has a tremendous advantage in the slaughterhouse, where superior carcasses can be selected and tissues taken for future cloning.

Another way in which cloning will impact the livestock industry is with genetically engineered donor cells. Through the various genome projects, the genomes of different organisms are being sequenced and the genes that impact different traits are being identified. As this information is made available, we will be able to add, remove, or modify genes of economic importance to the livestock industries in donor cells prior to cloning.

Research communities are also interested in producing cloned livestock. Using a uniform set of identical animals is ideal for conducting certain types of experiments. It removes genetic variability so that only the effects of the treatments or environment are measured. Therefore, it would be faster and easier to test, for example, new vaccines to determine the best treatment, reducing the number of animals needed to come to these conclusions. Additionally, researchers have found that livestock in some cases are better models for studying disease. Clones could be genetically engineered to mimic particular genetic disorders and compared to nonengineered clones so that only the gene causing the disease would be different. New treatments for the disease would then be more easily tested to determine which is the most effective.

Current Methods

Cloning first requires a supply of cells from the donor animal to be cloned. It is not yet clear which cells are the most effective for producing live cloned offspring. Currently, biopsies are taken either from fetuses or adults, essentially from any tissue in the body, and then propagated in culture to generate fibroblasts. It is critical for the practitioner to ensure that the area to be biopsied be thoroughly cleaned with disinfectant, and the tissue be collected using sterile technique in order to remove any chances of contamination, which could render the sample unusable. Tissue biopsies are transported to the lab, where they are minced and/or treated with enzymes and cultured to obtain cell lines. These cell lines can be expanded in large quantities and frozen in liquid nitrogen for future use. At the time of cloning, cells are removed from tissue culture plates by trypsin treatment and held in suspension until transfer.

Nuclear transfer involves removing the chromosomal DNA from mature oocytes and transferring the genetic material from a cell of the donor animal to be cloned through a process of fusion and reprogramming (Fig. 63-1). Briefly, immature oocytes are collected either from slaughterhouse ovaries or by transvaginal oocyte retrieval (TVOR) and matured in vitro as described above. After 18 to 22 hours, oocytes are stripped of their surrounding cumulus cells, and those with visible polar bodies (mature metaphase II oocytes) are selected for further manipulations. Using micromanipulators, each oocyte is enucleated by piercing the zona pellucida with a glass needle and removing the polar body with a small amount of the adjacent cytoplasm, containing the oocyte's chromosomal material, which is then discarded. A donor cell from the animal to be cloned is then transferred through the same hole in the zona of each enucleated oocyte. The donor cell is then fused to the enucleated oocyte by alignment in a fusion chamber and applying one or two DC pulses of 2.25 KV/cm, 15 μsec each, although voltage and timing may vary. Fused couplets are activated by either chemical or electrical stimulation in order to mimic sperm penetration and finish the reprogramming and maturation process. Reconstructed cloned embryos are then cultured in vitro for 6 to 9 days and evaluated for development. Viable compact morula and blastocyst-stage embryos are transferred to synchronized recipients

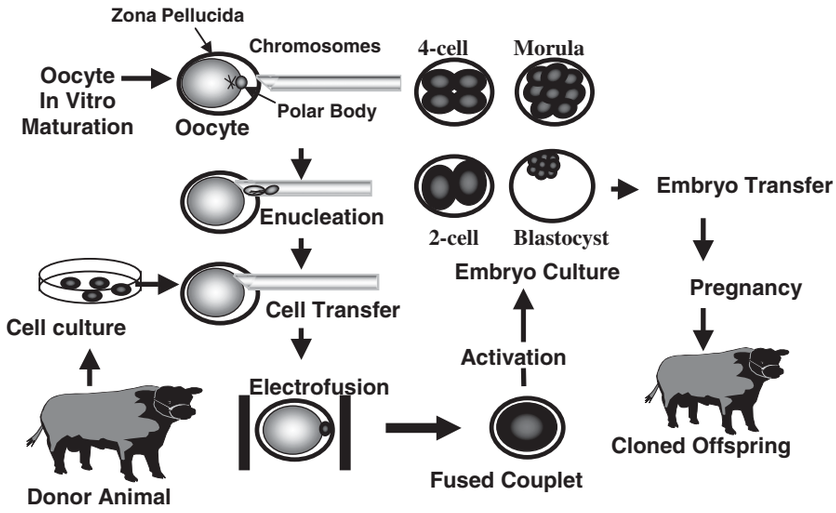

Fig. 63-1 General overview of procedures for producing a cloned calf.

as described above and carried to term in order to produce live cloned offspring. Additional care is required for recipients receiving cloned embryos and is addressed below. Cloning is now commercially available through several companies and universities.

Challenges in Cloning

Currently, the technology of nuclear transfer is quite inefficient. It is apparent that the inefficiencies of micromanipulation, with an average fusion rate of 64%, and an average blastocyst development rate of 33%, production of cloned embryos can still be improved upon. It is not clear yet which donor cell types give the best results; however, it is well established that younger passage cell lines yield more favorable results. Furthermore culture conditions of donor cell lines can greatly impact the survival rates of cloned embryos produced from them. There are additional factors that have an impact on the production of a live offspring that need to be addressed. These include early embryonic and fetal losses, which can occur any time during gestation. Losses occur most often between days 30 and 90 of gestation, and can be as high as 80%. On average, only 10% of cloned embryos transferred are carried to term, or less than 1% of the cloned embryos originally constructed. These issues are addressed below.

Another concern for nuclear transfer is the potential loss of genetic diversity. Although unlimited numbers of identical cattle can be produced with cloning, it should not replace natural breeding. If it did, it could result in loss of genetic variation and inbreeding, which are not desirable. This same concern was addressed when artificial insemination first became popular. Producers should be aware of these possibilities, and with proper guidance and management of breeding schemes, problems such as these will not occur.

CALVES FROM IVP AND CLONED EMBRYOS

The use of in vitro embryo production and cloning has stimulated considerable interest in the effects of reproductive biotechnologies on the offspring. The sex ratio of calves from conventional ET is not significantly different from that of calves resulting from AI. However, the selection and transfer of advanced stage embryos produced in vitro often results in slightly more bull calves. This may be attributed to the intriguing finding that male embryos tend to develop more rapidly in vitro than female embryos.

The birth weight of calves resulting from routine ET is not significantly different from that of calves resulting from AI. However, the transfer of embryos produced either in vitro using a variety of culture systems or by cloning has resulted in calves with normal to altered birth weight and development. An increase in calf birth weight of 15% or greater is often the most striking observation to a producer— hence, the term large offspring syndrome (LOS).[7,9-11] However, these calves can have other distinct developmental abnormalities involving the placenta, fetus, and/or calf, with or without an increase in birth weight.[12] Thus the term abnormal offspring syndrome (AOS) has been proposed to more accurately describe this syndrome.[12]

Commonly reported abnormalities associated with AOS include increased calf birth weight, hydrops of the allantois, reduced placental vascular development, increased abortions and dystocia, and increased perinatal death. For calves produced from cloned embryos, the increase in calf birth weight and other physical and biochemical abnormalities are often extreme. This condition has been reported in cattle, sheep, and mice. The biologic mechanisms that underlie this important condition remain to be uncovered. However, alterations in the expression of imprinted genes (e.g., IGF2 and IGF2 receptor), as well as nonimprinted genes (e.g., myostatin) most likely play a role.[10, 12]

Veterinarians attending to recipients carrying fetuses from in vitro–produced or cloned embryos need to regularly monitor the pregnancies by palpation and ultrasonography. Recipients carrying pregnancies from the transfer of these embryos should be given special attention at the time of calving because they may require obstetric assistance including cesarean section. Furthermore, neonatal calves may exhibit hypoxia, hypothermia, hypoglycemia, metabolic acidosis, and weakness.[9,12] Intensive neonatal care including administration of intravenous fluids and supplemental oxygen may be necessary to enhance calf survival rates.

In summary, in vitro production and cloning systems are effective for producing additional embryos and calves from donor cows and heifers. However, the practitioner should be prepared to monitor the pregnant recipient and address the problems that may occur in the recipient and the newborn calf. An important challenge facing IVP and cloning biotechnologies in cattle is the development of culture media that will consistently result in a higher percentage of viable blastocysts, normal pregnancies, and calves.

CONTROLLING SEX OF CALF

Embryo and semen technologies can be used alone or together to produce calves of the desired sex. This can be accomplished by determining the sex of the embryo before transfer, AI of single- or multiple-ovulated females with sexed sperm, using sexed sperm in in vitro embryo production systems, and by cloning.

Procedures for determining the sex of an embryo recovered from a donor cow or produced in vitro are well established. Briefly, a biopsy is obtained from the embryo mass using micromanipulation equipment, and then Y-chromosome-specific DNA within blastomeres is amplified using polymerase chain reaction (PCR). The sex of an individual embryo can be determined within hours with greater than 95% accuracy.[13] However, procedures for sexing embryos are expensive and require a high level of laboratory skill to perform. Biopsy of an embryo also can decrease the embryo's viability. In addition to determining sex of the embryo, biopsy and PCR may be used to detect specific traits of importance to health and production. For example, bovine leukocyte adhesion

deficiency (BLAD) can be detected in an embryo biopsy by PCR assay.[14]

Sexed bovine sperm is now commercially available and is an appealing technology for selectively producing heifer or bull calves. Sperm cells can be separated using a high-speed flow cytometer cell sorter into X- and Y-chromosome bearing populations. Spermatozoa are sorted through the equipment based on slight difference in DNA content; sperm cells containing X-chromosomes have about 4% more DNA. Approximately 10 million live sperm cells can be sorted by the equipment per hour with about 90% accuracy.[15] Insemination with sorted spermatozoa has resulted in the production of calves of the desired sex with about 90% accuracy (87.8% female calves from X-sorted sperm, and 92.1% male calves from Y-sorted sperm).[16] Thus, breeding programs can be targeted to produce calves with a wide array of specific purposes such as replacement heifers, bull calves for steer/beef production, and bull calves as potential AI sires. Field trials conducted with heifers inseminated with sexed semen demonstrated that pregnancy rates were 70% to 80% of that seen with unsexed semen. As with other reproductive technologies, breeding programs with sexed semen are more successful in well-managed herds. Based on data from more than 1000 calves, AI with sexed semen resulted in calves that did not differ in physical, health, or growth characteristics compared to calves from AI with unsexed semen.

Sexed semen can also be used to produce sexed embryos by insemination of superovulated cows or by in vitro fertilization of oocytes. The sexed embryos can then be transferred into synchronized recipients, as previously discussed. The production and transfer of embryos of known sex could potentially reduce the costs associated with conventional ET using unsorted semen. Currently, the limitations of sexed sperm technology in cattle are that expensive equipment is needed to sort sperm cells, lower efficiency of production per straw of semen, limited number of bulls available for selection as sires, higher cost per straw of sexed semen, and decreased fertility compared to unsorted sperm. Improvements in production efficiency and fertility of sexed semen could result in huge potential benefits for the dairy and beef industries.

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CHAPTER 64

Clinical Reproductive Anatomy and Physiology of the Buck

HARI O. GOYAL and MUSHTAQ A. MEMON

ANATOMY OF THE REPRODUCTIVE TRACT

The male reproductive organs include a pair of testes, which produce sperm and hormones; a pair of excurrent ducts (rete testis, ductuli efferentes, epididymis, and ductus deferens) where sperm mature and then are stored (in the tail of the epididymis) until ejaculated; a penis, which serves as a copulatory organ; and a set of accessory sex glands whose secretions (seminal plasma) form the bulk of the semen (Fig. 64-1).

The testes, although developed in the abdomen, migrate prenatally through the inguinal canal into the scrotum. The mature testes are ovoid in shape and positioned vertically in the scrotum; each testis can weigh 90 to 100 g in a 2- to 3-year-old Nubian buck with a 28 to 30 cm scrotal circumference.[1] The rete testis, a network of intercommunicating channels, occupies almost two thirds of the central axis of the testis. The ductuli efferentes, a group of 15 to 20 tubules, connect the rete testis with the epididymis, which, although a single tube about 60 to 80 m long, can be divided into three main regions: head, body, and tail. The head lies on the dorsocaudal border of the testis, the body is a thin strip-like structure attached on the caudomedial border of the testis, and the tail is an enlarged promontory structure located on the ventromedial border of the testis that can be palpated through the scrotum in mature goats. The ductus deferens connects the tail of the epididymis to the pelvic part of the urethra. Enwrapped by its own peritoneal fold, the ductus deferens ascends through the inguinal canal to enter the abdominal cavity at the vaginal ring. It then separates from the other parts of the spermatic cord and pursues a course via the genital fold to the caudal part of the bladder. The distal part of the ductus deferens enlarges to form the ampulla, which is 6 to 7 cm long and 4 to 5 mm in diameter, and dips under the prostate to open with the excretory duct of the seminal vesicle on the side of the colliculus seminalis.[2]

Seminal vesicles, the largest of the accessory sex glands, are elongated saccular organs with a lobulated surface. They lie on each side of the caudal part of the dorsal surface of the bladder. Each gland in a 2- to 3-year-old Nubian buck is about 4 cm long, 2.5 cm wide, and 1.5 cm thick.[2] Although they lie in close contact with the rectum, it is difficult to palpate them through the rectal wall. Each excretory duct passes through the prostate gland and opens on the colliculus seminalis. The entire prostate gland is disseminated and completely surrounds the wall of the pelvic urethra. Numerous small ducts carry the prostatic secretion into the urethra. The paired bulbourethral glands lie on the dorsal surface of the urethra opposite the ischial arch and are covered by a thick layer of dense connective tissue and by the proximal part of the thick bulbospongiosus muscle. The excretory duct of each gland opens into the urethra under a blind fold of mucous membrane, which makes it difficult to pass a catheter into the bladder.

The penis is classified as fibroelastic because it is covered by thick fibrous tissue with relatively poorly developed venous sinuses. It is rigid even in the quiescent stage. Its body forms a sigmoid flexure, which is located caudal to the spermatic cord. During erection, the sigmoid flexure straightens and the elongated penis becomes rigid without the erectile tissue undergoing much engorgement. The glans penis is a somewhat rounded cushion of erectile tissue. The urethral process is peculiar because it projects beyond the glans penis by about 2 to 3 cm.

SPERMATOGENESIS AND TRANSPORT, MATURATION, AND STORAGE OF SPERM

Spermatogenesis

Spermatogenesis is a highly synchronized and hormonally controlled sequence of events wherein the germ cells undergo a series of divisions and differentiation (spermatogonia, primary spermatocytes, secondary spermatocytes, early spermatids, and late spermatids) resulting in the formation of haploid sperm. Mathematically, 64 sperm cells can result from one spermatogonium, and in sheep, the entire cycle of spermatogenesis, from the first spermatogonial division to the release of sperm into the lumen of the seminiferous tubule, is completed in 49 days.[3] The cycle is expected to be similar in goats. Spermatogenic cycling begins with the onset of puberty and is repeated at a fixed interval throughout the life of the animal.

The differentiation of spermatogonia to sperm is closely regulated by Sertoli cells, which form close junc-

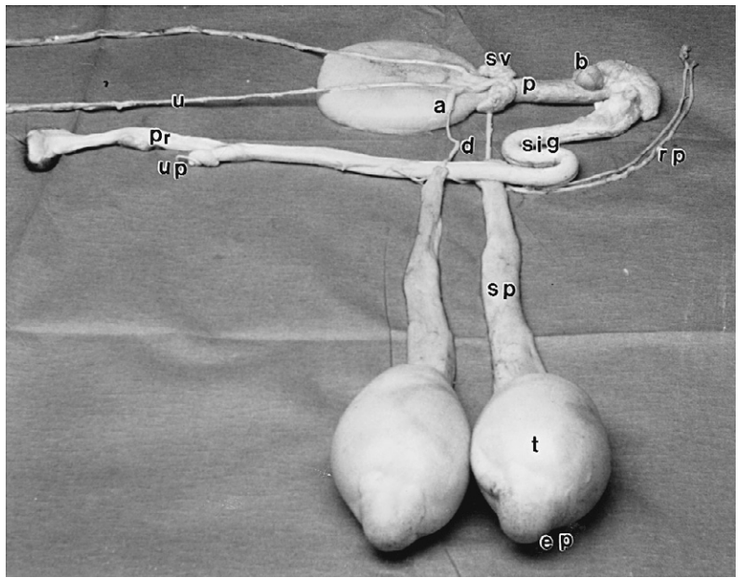

Fig. 64-1 Genital organs of a normal buck. a, ampulla; b, bulbourethral glands; d, ductus deferens; ep, tail of the epididymis; p, prostate gland; pr, prepuce; rp, retractor penis muscle; sig, sigmoid flexure; sp, spermatic cord; sv, seminal vesicle glands; t, testis; u, ureter; up, urethral process.

tional contacts with germ cells. In addition to Sertoli cell secretions, testosterone is essential for spermatogenesis in all mammalian species, including goats. In fact, spermatogenesis requires a 50 to 100 times higher level of testosterone surrounding the germ cells than in the systemic circulation.[4] Testosterone is also essential for production of seminal plasma, epididymal maturation of sperm, and development and maintenance of secondary sex characteristics and libido. More than 90% of the body's testosterone is secreted by the Leydig cells, whose structure and function depend on the functional maturity of the hypothalamus-pituitary–Leydig cell axis. The hypothalamus secretes luteinizing hormone–releasing factor, which stimulates the pituitary to secrete luteinizing hormone, which, in turn, stimulates the Leydig cells to secrete testosterone. The body maintains a homeostatic level of testosterone through the negative feedback effects of testosterone on the pituitary gland or the hypothalamus or both.[4]

Transport

After leaving the seminiferous tubules, the maturing sperm pass through the rete testis, the ductuli efferentes, the head of the epididymis, the body of the epididymis, and into the tail of the epididymis, which serves primarily as a storage site until ejaculation. Sperm passing through these ducts are immotile, so their transport is accomplished primarily by peristaltic contractions of smooth muscle cells surrounding the ducts. Transport is completed within 10 to 15 days.[5]

Maturation

While passing through the excurrent ducts, especially the epididymis, sperm undergo maturational changes that enable them to acquire the abilities to move in a forward direction and to enter and fertilize an ovum. Although the mechanisms responsible for sperm maturation are not fully understood, it is generally believed that certain epididymal secretions, principally glycoproteins, coat the cell surfaces of immature sperm and thus cause them to undergo a functional change.[6,7] The secretion of epididymal proteins is androgen-dependent, and any long-term disturbance in their synthesis or secretion can affect fertility.

Storage

Sperm are stored in the tail of the epididymis and can stay there without losing motility or fertility for 5 to 14 days in primates[8] and substantially longer in other species of domestic animals.[5] The epididymal capacity to store sperm has a direct effect on sperm concentration per ejaculate and on sperm depletion. In our experience, semen from 2- to 3-year-old Nubian bucks can be collected every other day for several weeks without any significant decrease in total number of sperm per ejaculate. According to Amann and Schanbacher[9] the number of sperm stored in the tail of the epididymis is maximal in rams that have not ejaculated for at least 7 to 10 days but is reduced by at least 25% in males ejaculating daily or every other day.

REPRODUCTIVE PHYSIOLOGY

Puberty

The onset of puberty can be defined as a gradual progression of a number of biologic events, such as the onset of spermatogenesis, appearance of sperm in the ejaculate, and capability of intromission. The pubertal age is breed-

dependent, varying from 2 to 3 months in Milch bucks[10] to 4 to 5 months in Nubian, Moxoto, and Boer bucks,[11–13] to 12 to 48 months in Damascus bucks.[14] Most breeds of goats raised in the temperate environment of the Northern Hemisphere possess sperm in the ejaculate at 4 to 5 months. However, at this age, their semen quality is poor, and therefore, they should not be used for breeding. Nubian bucks start producing quality semen at about 8 months of age and begin exhibiting libido, characterized by the desire of young bucks to mount other animals, at 10 to 12 weeks.[13]

Whether kidding season (winter versus summer) can influence puberty remains unknown, but poor management, such as overcrowding, early weaning, and low-quality feed, and multiple births (double or triplet versus single) can delay the onset of puberty. One common feature that is noticed in all prepubertal bucks is the presence of attachments between the prepuce and penis. In Nubian bucks, these attachments begin to separate at 3 months. The urethral process is separate at 3.5 months and the glans penis is completely free of the preputial mucosa by 4.5 months. Because fertile matings are possible in 4- to 5-month-old bucks, males should be separated from does by 4 months of age, or earlier for fast-growing kids, especially those born as singlets, to prevent accidental breeding.

Sexual Behavior

Sexual behavior of bucks can be described as a pattern of well-defined steps, including actively seeking estrous females, courtship, mounting, intromission, and ejaculation. After detecting an estrous female, the buck enters into courtship behavior, which entails kicking, pawing, and nuzzling the female, grunting, and displaying the flehman response (curling of the upper lip). Once the female stands motionless, the buck attempts one or two false mounts, which are followed by mounting with intromission of the penis into the vagina. Ejaculation occurs spontaneously and is characterized by a strong pelvic thrust with a rapid backward movement of the head. After ejaculation, the buck dismounts and displays refractoriness (no sexual arousal) for a few minutes to a few hours. Sexual behavior is androgen-dependent and its onset in prepubertal animals coincides with a rise in testosterone levels.[15] Other factors that may influence sexual behavior are season, social contact (presence of a dominant buck), nutrition, disease, and stress.

FACTORS AFFECTING SEMEN PRODUCTION

Age

Immature bucks of all breeds produce poor-quality semen. Nubian bucks by 8 months of age begin to produce high-quality semen with sperm motility and morphology as good as that of 1- to 5-year-old bucks. However, the semen volume, sperm concentration per milliliter, and total number of sperm per ejaculate increase gradually up to 2 years of age.[1] Similarly, in Alpine and Poitevine bucks, the number of sperm produced in the first breeding year represents only 60% of that produced in the second breeding year.[16] Whether old age (5 years and after) has any effect on some or all of the seminal attributes has not been adequately studied.

Photoperiod (Season)

Whether bucks are truly seasonal breeders remains controversial. Semen from Nubian bucks raised in a temperate environment and trained at an early age can be collected with an artificial vagina throughout the year without any noticeable change in sexual behavior or seminal characteristics.[13] On the other hand, studies conducted in different parts of the world have found varying degrees of seasonal effects on some or all seminal attributes.[17,18]

Temperature

Spermatogenesis in most mammals (except the elephant and whale) requires a temperature 3° to 5°C lower than body temperature. The body's failure to maintain lower testicular temperature such as that resulting from high ambient temperature, high fever, frostbite of the scrotum, and retention of testes within the abdominal cavity invariably affects seminal quality, depending on the duration and intensity of the heat. However, if the causative influence is corrected in time, normal semen production can be restored.

Nutrition

Poor nutrition in general, and protein deficiency in particular, are known to have a deleterious effect on the general health of animals of all age groups. Noteworthy effects on reproduction might include delayed puberty and sexual maturity, diminished libido, and decreased or even complete loss of spermatogenesis. Generally these effects are mediated through the central nervous system, and more specifically through the hypothalamic-pituitary-testicular axis. Conversely, the deficiency of certain vitamins (A, B, and E), amino acids (arginine, lysine, tryptophan, phenylalanine, and histidine), and minerals (zinc) affects the testes directly and can cause variable impairment of spermatogenesis.

Disease

Certain common diseases that are known to have a deleterious effect on seminal quality are varicocele, unilateral or bilateral cryptorchidism, epididymitis, and spermatocele. In addition, genetic disorders such as XXY syndrome, intersex or hermaphroditism, and hypospadias (incomplete development of the prepuce) have been reported to occur in bucks of some breeds.[19] Interestingly, genetically related sperm abnormalities including the Dag defect, the diadem defect, the corkscrew defect, and the pseudodroplet defect, which have been related to subfertility in bulls, have not yet been reported in goats.

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CHAPTER 65

Examination of the Reproductive Tract and Evaluation of Potential Breeding Soundness in the Buck

MUSHTAQ A. MEMON, W. DUANE MICKELSEN, and HARI O. GOYAL

PHYSICAL EXAMINATION

A physical examination must include a general examination for health with special consideration of the reproductive organs. To be a satisfactory potential breeder, a buck should be in good flesh. Thin or excessively fat animals should be avoided. A little reserve flesh is desirable, since bucks can be expected to lose weight during the breeding period. Bucks with severe structural faults or diseases of hindlimbs will be less inclined to serve females. One common structural fault is the postlegged condition, in which the hindlimbs are too straight. The result is usually increased pressure on the pasterns and abnormal growth of the hooves, which require frequent trimming. Arthritic conditions can occur. Bucks should be examined for foot rot and foot abscesses.

EXAMINATION OF THE REPRODUCTIVE TRACT

The testes should be examined for size, symmetry, and consistency (Fig. 65-1). Seasonal variations and breed differences must be kept in mind. A sound buck during the breeding season should have large oval testes that are firm to the touch and of equal size. Soft, nonresilient testes are usually seen during the nonbreeding season or may be associated with poor health. Scrotal thermography and ultrasonography are techniques that may be useful in aiding detection or confirmation of abnormalities of the scrotum and its contents. These recently developed diagnostic methods are not yet economical or practical in field situations. The epididymis consists of three anatomic parts: the head, the body, and the tail. Attention should be given to alterations in their size, form, and consistency. Gross alterations in the epididymis are fairly rare in the goat.

The penis should be extended and examined. This is usually accomplished while collecting a semen sample when an electroejaculator is used. Otherwise, the penis must be manually extended from the sheath so that a careful examination can be made. The urethra extends beyond the tip of the penis about 2 to 3 cm, forming the urethral process. The urethral process is thought to aid in spraying semen around the os of the cervix. When bucks have urinary calculi, the urethral process is the common area of obstruction. The urethral process is usually removed during treatment of urolithiasis with no apparent detrimental effect on fertility.[1]

SCROTAL CIRCUMFERENCE AND ITS CORRELATION WITH SPERM PRODUCTION

Scrotal circumference measurements, because of their high correlation with testicular size and capacity for sperm production, have become an integral part of the breeding soundness evaluation of bulls. Information on similar relationships is limited in bucks, however. Using a nonstretchable tape, the examiner measures the scrotum at the point of its greatest diameter, pulling the tape snugly and applying moderate tension so that the testes are pulled together and the tape is in contact with the entire circumference (Fig. 65-2). There is a positive correlation between scrotal circumference and body weight in bucks (Table 65-1).[2] Based on data collected in young growing and mature Nubian bucks, the mean scrotal circumference measurements at 5, 8, 21, and 36 months of age are 14, 20, 26, and 30 cm, respectively. In mature bucks, scrotal circumference is positively ($P < 0.01$) correlated with both sperm concentration per milliliter and total sperm per ejaculate, but the correlation is significantly higher in the latter (r, 0.66 versus 0.36).[3]

LIBIDO

Libido is an essential component of breeding performance but is difficult to measure during a routine breeding soundness examination. When semen samples are collected with an artificial vagina, libido can be assessed to some degree. A carefully obtained history may reveal

TABLE **65-1**

Body Weights and Scrotal Circumference of Bucks*

	BODY WEIGHT, kg		SCROTAL CIRCUMFERENCE, cm	
BUCKS, *n*	***Mean* ± *SD***	***Range***	***Mean* ± *SD***	***Range***
13	4.4 ± 0.2	≤4.9	6.8 ± 1.7	4.9–7.9
20	7.6 ± 1.4	5.0–9.9	11.0 ± 3.2	7.1–17.5
12	12.0 ± 1.2	10.0–14.1	15.9 ± 3.1	10.5–19.8
15	17.3 ± 1.8	15.0–19.9	18.3 ± 2.9	11.9–21.0
16	22.0 ± 1.7	20.0–24.9	20.8 ± 1.7	16.2–22.3
10	27.5 ± 1.0	25.0–29.9	21.5 ± 2.1	17.7–24.4
10	32.7 ± 1.4	30.0–34.9	23.3 ± 2.6	19.5–26.8
13	37.3 ± 0.9	35.0–39.9	25.0 ± 1.7	23.0–27.0
13	43.9 ± 3.4	≥40.0	26.4 ± 1.8	24.8–28.4

*Means of combined results from 122 crossbred bucks.
From Bongso TA, Jainudeen MR, Zaharah AS: Relationship of scrotal circumference to age, body weight, and onset of spermatogenesis in goats. *Theriogenology* 1982;18:513; with kind permission of Elsevier Science-NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands.

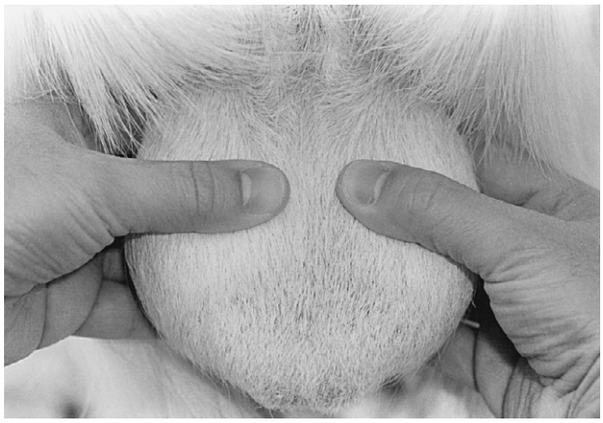

Fig. 65-1 Evaluation of testicular consistency. Testes are held in both hands and pressure is applied with both thumbs.

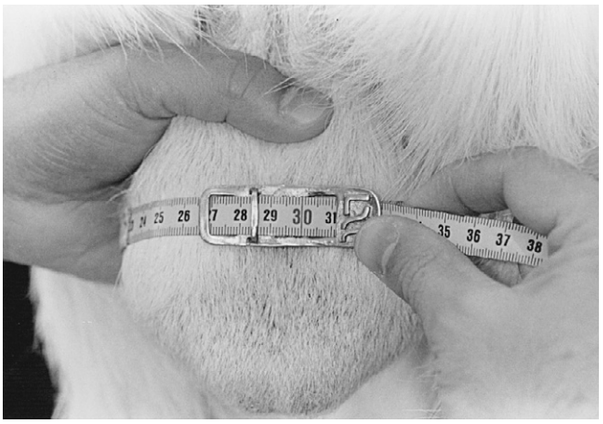

Fig. 65-2 Scrotal circumference is measured by applying the measuring tape snugly at the widest part of the testes with one hand while holding both testes down with the other.

whether does are being covered and whether intromission and ejaculation (indicated by a sudden backward motion of the head) occur.

SEMEN COLLECTION

Semen can be satisfactorily collected from bucks using an artificial vagina (AV), an electroejaculator, or a Bailey ejaculator* (Fig. 65-3). The AV consists of the following parts: (1) a rubber tube that is 8 inches long and 2 inches in diameter and is equipped with an air and water valve, (2) a rubber pressure bulb, (3) a latex inner liner, (4) a latex semen collection funnel, (5) two wide rubber bands, (6) a 15-ml graduated Pyrex collection vial, and (7) a nondrying, nonspermicidal lubricant.† No exogenous stimulus is needed when semen is collected with an AV; however, the buck must be trained to serve the AV using a mount animal. A doe in estrus is an ideal mount animal; however, a doe in diestrus injected intramuscularly with 5 to 10 mg prostaglandin F_2 alpha,* or an ovariectomized doe injected intramuscularly on alternate days with 1 mg of estradiol cypionate† could be used as a mount animal.[4] The casing of the AV is filled with warm water to provide an internal temperature of approximately 39° C at the time of collection. Air is added to create pressure and the AV is lubricated immediately before collection. After two false mounts, the buck is allowed to serve the AV. The penis is never handled directly, but is directed by grasping the sheath or the prepuce. The buck will seek the AV, then thrust into it. The AV should never be slipped onto the penis.

Bucks are restrained in a chute or held against a wall for semen collection with the Bailey ejaculator or electroejaculator. The electroejaculator rectal probe with

*Western Instrument Co., Denver, CO.
†Nasco Farm and Ranch Company, Fort Atkinson, WI.

*Lutalyse, The Upjohn Co., Kalamazoo, MI.
†ECP, The Upjohn Co., Kalamazoo, MI.

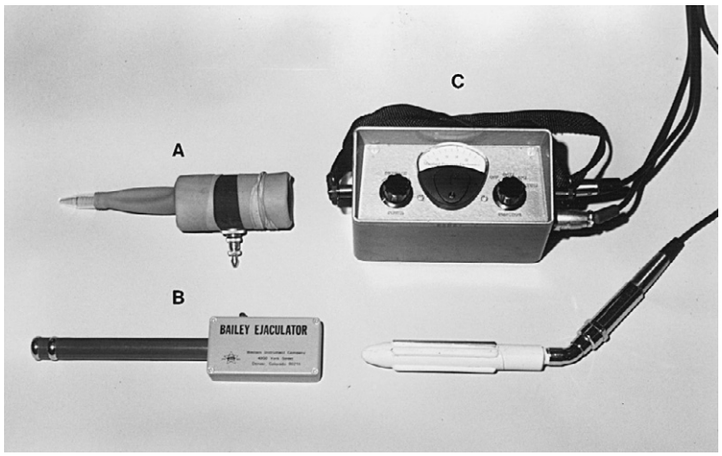

Fig. 65-3 Artificial vagina **(A)**, Bailey ejaculator **(B)**, and electroejaculator **(C)** for semen collection from the buck.

three electrodes (19 × 3.5 cm, length × diameter) is lubricated and inserted into the rectum. Intermittent stimulation is obtained by rotating the variable power knob to up to three and then back to zero at a frequency of once every 2 to 3 seconds. During and after collection, semen should be protected from direct sunlight and temperature shock, and sperm motility should be evaluated within 10 minutes of collection.

The use of an electroejaculator or a Bailey ejaculator has one disadvantage—libido cannot be assessed because of the forced ejaculation. In our experience a greater volume of semen but a lower concentration of spermatozoa was collected with the electroejaculator and Bailey ejaculator methods than with the AV method.[4] Also, the use of the electroejaculator, and especially the Bailey ejaculator, resulted in increased vocalization and excessive muscular contraction of the hind limbs. Owing to this undesirable response, the Bailey ejaculator in the present form (11.2 volts), is not recommended for semen collection in goats.[4] A technique used successfully and with very little trauma to the buck is massage of the rectum for 30 seconds with the rectal probe of the electroejaculator and then use of direct stimulation for 8 to 10 seconds. Usually, the semen sample is collected with one stimulus, but if the buck fails to ejaculate, one must wait an additional 30 to 60 seconds and then repeat the stimulus one more time.

SEMEN EVALUATION

Many factors including semen volume, motility, concentration, and morphology of spermatozoa have been used for assessing the quality of semen. No single test has been developed that is an accurate predictor of fertility of individual ejaculates. Both semen quantity and quality can vary with age, season, temperature, breed, and even between individuals within the same breed. Generally, semen from goats has a low volume and a high concentration of spermatozoa (Table 65-2).

Semen evaluation for the purpose of breeding soundness examination should include the following parameters.[5]

TABLE **65-2**

Normal Values for Semen of Adult Bucks*

Measurement Parameter	Normal Value
Volume, ml	1.0 (0.5–1.5)
Motile sperm, %	80 (70–90)
Sperm concentration, 10^9/ml	4 (2–5)
Morphologically normal sperm, %	80 (70–90)

*Values given as mean (range).
Adapted from Memon MA: Male infertility. *Vet Clin North Am Large Anim Pract* 1983;5:539; and Skalet LH: Effects of age and season on the spermiogram of Nubian male goats. MSc Thesis, Tuskegee University, Tuskegee, AL, 1986.

Volume

The volume is measured in milliliters directly from the graduated collection vial. This parameter is reliable only for ejaculates collected with an AV.

Color

The color of semen depends on the number of sperm per milliliter and can vary from whey-like ($<0.1 \times 10^9$/ml) to milky ($0.5–1.5 \times 10^9$/ml) to creamy ($2.5–\geq 4 \times 10^9$/ml).

Gross or Mass Motility

A small (~10 µl) drop of fresh, undiluted semen is placed on a prewarmed (37°C) glass slide without a coverslip, and the wave motion is observed using a 10× phase-contrast objective (magnification ×100). An estimate of the vigor of the wave is graded as follows: very good (++++, vigorous swirls), good (+++, slow swirls), fair (++, no swirls, but generalized oscillation), or poor (+, sporadic swirls).

Progressive Motility

The percentage of progressively motile spermatozoa is one of the most widely used tests for semen quality. A drop of fresh semen is diluted with prewarmed, phosphate-buffered saline or 2.9% sodium citrate solution (pH 7.4). A small (~10 µl) drop of the diluted semen is placed on a prewarmed slide, covered with a prewarmed coverslip, and then examined with a 40× phase-contrast objective (magnification ×400) using a microscope equipped with a thermocontrolled heating stage set at 37°C. Five fields are randomly observed and the number of spermatozoa moving in a forward direction are subjectively estimated (nearest 5%) in each field. Circular or reverse motion is often a sign of cold shock or chemical shock. However, a reverse motion may also be seen if there is a high percentage of midpiece abnormalities.

Concentration

Concentration is not routinely measured in field conditions. In laboratory situations, concentration is calculated by use of a hemocytometer for occasional samples and by use of a calibrated spectrophotometer for large numbers of samples. The concentration of sperm is expressed as the number of sperm per milliliter and total number of sperm per ejaculate. The latter is calculated by multiplying the former with the volume of the semen. Sperm concentration can be measured by a commercial Unopette system.* The semen sample is stirred gently to create a uniform suspension. The semen is drawn up into the capillary pipette (20 µl) provided with the kit and dispensed into the second diluent container according to the kit instructions. The diluted semen is loaded into the two counting chambers of a hemocytometer, and sperm are counted at ×100 magnification. The number of sperm in the large center square equals the number of million sperm per milliliter in the ejaculate. The total number of sperm in the ejaculate equals the concentration (sperm per milliliter) multiplied by the ejaculate volume.

Morphology

Semen from bucks contains some abnormal spermatozoa, but this is not usually associated with lower fertility until the percentage of abnormal spermatozoa is greater than 20%. The types of abnormalities can be counted by examining stained or unstained (air-dried) semen smears. The commonly used stains are eosin-nigrosin, Wright's, and Williams' stains. Staining with new methylene blue is frequently used to detect white blood cells, which are indicative of infections.

Generally, sperm abnormalities are evaluated in an eosin-nigrosin–stained smear with a bright field microscope or in an unstained smear with a phase-contrast microscope. A small droplet of fresh undiluted or diluted semen (1:10), depending on the concentration of sperm, is placed on a glass slide and mixed with a drop of eosin-nigrosin stain (if a stained smear is to be used); the smear is prepared by drawing a coverslip across the slide. The smear is air-dried, and 200 sperm per slide are examined using a 100× objective (magnification ×1000) and classified as normal or as primary or secondary abnormalities. Primary abnormalities include (1) misshapen heads (microcephalic, macrocephalic, round, elongated, pyriform, swollen, missing), (2) abnormal acrosomes (missing, swollen, folded), (3) abnormal middle pieces (abaxial, double, swollen, coiled, kinked, bent, looped), (4) abnormal principal pieces (coiled, looped), (5) fractured necks, and (6) proximal protoplasmic droplets. Secondary abnormalities are (1) distal protoplasmic droplets, (2) bent principal pieces, and (3) detached normal heads.

*Becton-Dickinson, Rutherford, NJ.

CONCLUSIONS

A satisfactory potential breeder is a buck that can be expected to produce a high conception rate in a group of sound, disease-free, eligible does. The following minimum values for semen collected with an AV from a satisfactory potential breeder are suggested: semen volume 0.5 ml, motile sperm 70%, sperm concentration 2 10^9/ml, morphologically normal sperm 80%. A questionable potential breeder may require re-examination at a later date. A buck declared questionable would not make an acceptable candidate for purchase or sale. Unsatisfactory potential breeders are not necessarily infertile or subfertile, but they may be rejected for reasons other than semen quality. Examples are a buck with a unilateral cryptorchid testis or a buck with hindlimb impairment.

The rating given as a result of the examination is a scientific opinion or a judgment of the veterinarian. The results of a breeding examination are valid only for the day of the examination and do not ensure continued fertility. Owners, of course, will be interested in a judgment of prior and potential reproductive performance of the buck and information concerning the etiology of any discovered malady. Testicular degeneration and hypoplasia are not uncommon in bucks, but assessment of causative factors and prognosis is difficult. With testicular degeneration, the prognosis is grave; however, animals afflicted with a transient disease or environmental stress exhibit poor semen quality for variable periods of time and may recover full breeding soundness. Therefore, bucks classified as questionable or unsatisfactory breeders should be re-evaluated after an 8-week interval. Only those bucks that are consistently classified as unsatisfactory potential breeders should be culled.

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CHAPTER 66

Infertility and Diseases of the Reproductive Organs of Bucks

W. DUANE MICKELSEN and MUSHTAQ A. MEMON

Reproductive efficiency is important in dairy goat herds, and selection of highly fertile buck goats is absolutely essential. Many problems exist that may cause infertility in bucks; they are discussed here in some detail. Hand-mating is the usual practice in dairy goat herds, for it is not desirable to allow odorous bucks to run free with lactating does. The duration of estrus in does is 32 to 40 hours, and does are usually mated at 12-hour intervals until estrus subsides. Does actively seek the presence of the buck when in estrus and, when given a choice, usually prefer a scented buck to a deodorized one. Bucks can initiate and even synchronize cyclic activity in does at the beginning of the breeding season. Detection of estrus in does is best accomplished with the use of the buck. An intact buck should be penned in an area where does in estrus can be observed congregating near the pen.

LIBIDO

Mounting and thrusting behavior, sniffing the urogenital region, and exhibiting the flehman response are common behaviors of adult bucks. As in sheep, during the nonbreeding season (in the Northern Hemisphere, autumn is the breeding season), it may be difficult to obtain an ejaculate with an artificial vagina or the buck may be very slow to serve a doe in heat.[1] Out of the breeding season the buck may rest his head on the doe's hindquarters but fail to align himself with the longitudinal axis of the doe.[2] With the onset of the breeding season, bucks properly align, and mounting, thrusting, and ejaculation occur.

One of the most common reasons for a buck to come to the end of his breeding career is arthritis. Painful hips, stifles, or hocks cause reluctance or inability to mount. If several bucks are run together with does, a dominant but arthritic male may prevent breeding by another fertile buck. Lame bucks also tend to lose body condition because they do not get up to eat as much as they should. The cause of lameness may be trauma (degenerative) or infectious. Bucks with degenerative arthritis, which is often caused by congenitally poor conformation, or with other anatomic defects, should not be used for breeding.

Infectious causes of arthritis may result from caprine arthritis encephalitis (CAE) virus. With the CAE virus, the clinical disease is more common in some families than in others, suggesting an inherited susceptibility to effects of the virus. Research has shown that clinical CAE is associated with certain histocompatibility antigens, again suggesting a hereditary component.[3] Transmission of CAE infection by breeding appears to occur infrequently, if at all.[4] Detection of antibodies against CAE virus at the Washington Animal Disease Diagnostic Laboratory is performed with the use of a kinetic enzyme-linked immunosorbent assay. The antigen used in this assay is prepared from purified CAE virus with a technique designed to enhance the dominant antigens against which goats infected with CAE develop antibodies.[5]

INTERSEXES

Abnormal sexual differentiation leading to the intersex condition is relatively common in goats. It is more prevalent in dairy goats of the Saanen, Toggenburg, and Alpine breeds than in any other breeds of animals.[6] Intersexes exhibit phenotypic variations ranging from nearly normal females to nearly normal males. Generally they are female-like at birth, but as they reach the age of sexual maturity, they become larger than normal females, with a masculine head and erect hair on their neck. They exhibit small teats and a bulbous clitoris or a shortened penis. At puberty the clitoris becomes enlarged enough to be externally visible.[7]

The intersex acts like a female at birth but later, usually at puberty, starts to butt like a buck and become aggressive toward other goats and people. These animals also develop the odor characteristic of bucks. A majority of intersexes show pronounced male libido in the presence of a doe. Some of the more female-like intersexes lactate.[6] Intersexes are named with reference to the gonads they carry. The term "true hermaphrodite" is used to distinguish an animal that carries both gonads from those that carry either one or the other type of gonads (pseudohermaphrodites). Male pseudohermaphrodites have testes and female pseudohermaphrodites have ovaries. Most intersexes, however, have testes. Usually the testes are abdominal or they may be partially or totally descended (Fig. 66-1). The principal hormone produced by the gonads in caprine intersexes is usually testosterone.[6] Intersexes are frequently seen in polled dairy goats. The most accepted hypothesis is that hermaphroditism and horned traits are controlled by recessive genes and that these two loci are close to each other on the same chromosome (linked). The polled condition is the result of a mutation at the horn locus. The polled trait (P) is domi-

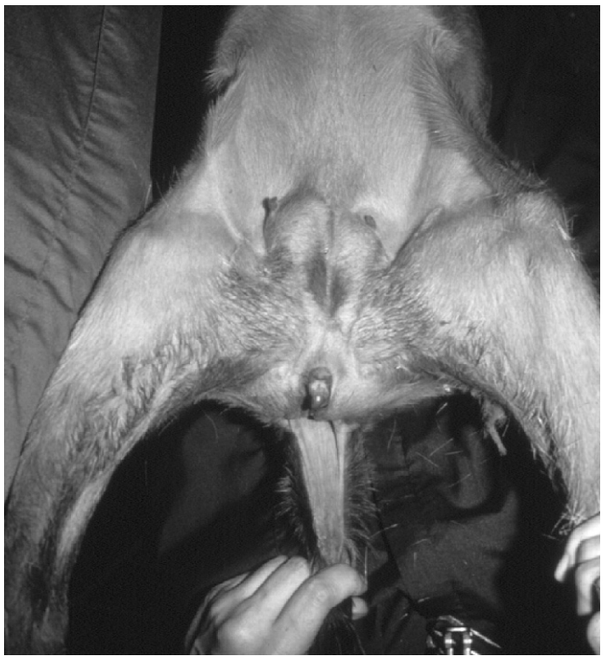

Fig 66-1 Hermaphrodite goat, on its dorsum, showing testes, vulva, and enlarged clitoris.

nant to the horned trait (p) and appears together with hermaphroditism (h) in PPhh or Pphh goats because the two loci are linked.[8] Therefore, intersexes will be seen mainly among polled goats and only rarely among horned animals (pp) because of the occasional crossing over between the two loci, which results in a pphh genotype. Cytogenetic evaluations of caprine intersexes clearly show that most polled intersexes are karyotypically female (XX) and the breeding histories of the parents indicate that intersexes are homozygous for the polled trait.[9] The presence of male gonads in these homozygous polled (PP) intersexes of XX makeup suggests that they are sex-reversed females. In the female caprine fetus, which is homozygous for the polled gene, two doses of the P gene divert the process of sexual differentiation toward the male despite the presence of two X chromosomes.[10]

Intersex goats have been found to have the H-Y antigen. The H-Y antigen is a male-specific transplantation antigen and is reportedly lower in intersexes than in normal XY males.[11] The suggestion has been made that animals of the same genotype and sex may differ in the density of H-Y antigen on their cell surface, which might explain the variability in primary sex determination among goats.

The gene for the polled trait has an impact on the reproduction performance of bucks. Bucks that are homozygous for the polled gene tend to become sterile. Although the testes of these bucks are of normal size and the seminiferous tubules display active spermatogenesis, there are no sperm detectable in the ejaculates because of blockage in the caput epididymidis. These trapped sperm form hard masses of variable size that are palpable near the head of the epididymis. In older bucks, the seminiferous tubules are closed, causing degeneration or rupture, releasing sperm into the interstitium (extravasation), which often leads to sperm granuloma formation.[12] Thus, the homozygous state for the polled gene is disadvantageous to both sexes. In males, it causes poor differentiation of the duct system, resulting in sterility, and in genetic females it causes gonadal reversal leading to masculinization of the gonads and external genitalia.

Freemartinism

The incidence of twins or triplets is higher than that of single births in goats. Perinatal mortality rate is lowest in twins compared with singlets or triplets. However, the incidence of intersexes is higher in twins and triplets than in singlets. A prerequisite for caprine freemartinism is birth as a twin to a male kid or as one of heterosexual multiples. Sometimes birth as a twin may not be recognized if one of the twins dies in utero. In these cases, confirmation of freemartinism can be obtained from chromosome analysis, which reveals the coexistence of male and female cells in the blood and other hematopoietic tissues. In caprine freemartins, the proportion of male cells in leukocyte culture could be as low as 1%, which necessitates careful examination of a number of cells for an accurate diagnosis of the freemartin condition.[13] Because twinning occurs in horned and polled goats, freemartin intersexes can be seen among both types of goats. Approximately 6% of intersexes can be expected to be freemartins. It is believed that in a majority of twin pregnancies in goats, vascular anastomosis does not occur similar to that in the bovine species. The external and internal genitalia of caprine freemartins are similar to those of polled intersexes. However, the masculine features are generally more exaggerated, and the gonads are partially descended testes devoid of germ cells.[13] Intersexes and freemartins do not produce sperm, even during the breeding season, and generally are less odoriferous than normal bucks.

Total Chimeras

Chimerism may result from fusion or cellular admixture of male and female embryos at an early stage in embryogenesis or from fertilization of the second polar body and ootid by X- and Y-bearing spermatozoa prior to fusion of the polar body with the ootid.[13] Although the occurrence of this type of intersex is rare in goats, approximately 1% of caprine intersexes belong to this category. Some of these are true hermaphrodites with a nearly normal ovary on one side and a testis or ovotestis on the other. Karyotypic analysis shows that they are whole-body chimeras.

TESTICULAR AND EPIDIDYMAL ABNORMALITIES

Testicular Degeneration

On digital palpation, normal testes feel resilient, with a consistency as firm as muscle. The ultrasonographic

appearance of a normal testis is one of uniform homogenicity with a central hyperechoic line representing the mediastinum testis. The testicular tunic and capsule are also distinct hyperechoic lines, whereas fluid around the testes (hydrocele) is hypoechoic.[14] The tail of the epididymis is more heterogeneous and less echogenic than the testis.[15] Testicular degeneration, or atrophy, of unknown etiology but accompanied by abnormal spermatozoa appears to be common in some goat populations. Often the testes are more elongated and smaller than normal. The head of the epididymis may undergo a palpable loss of lobulation. Multiple foci of calcinosis are grossly visible on the cut surface of the testis.[16] Ultrasonographically, testicular degeneration and mineralization are hyperechoic.[17] Firm, mineralized parenchyma, detectable by ultrasonography, has been observed in older or infertile bucks.[18] Testicular degeneration or atrophy has been found in a number of clinical conditions including systemic disease, epididymitis, debility from various causes such as poor nutrition and parasitism, aging, and higher or lower than normal environmental temperatures.[19]

Testicular Hypoplasia

Abnormally small testes may be found in animals suffering severe malnutrition or in animals that are intersexes. Hypoplasia is often difficult to distinguish from atrophy, but with either, testicular size and function should improve with proper feeding if malnutrition is responsible.[19] Hypothyroidism and severe zinc deficiency compounded by reduced feed intake have resulted in decreased weight of the testis and epididymis, a reduction in spermatogenesis, and degenerative changes in the testes and accessory sex glands.[20,21] Intersexes and freemartins often do not undergo the testicular size increase that normally occurs at puberty, or else the testes may atrophy at that time. One case of testicular hypoplasia has been reported in a horned buck with chromosomal mosaicism (XXY and XY).[22]

Cryptorchidism

Cryptorchidism is a developmental abnormality in which one or both testes fail to descend from the abdominal cavity into the scrotum. The retained testis may stop along the normal path of descent, or it may divert to an ectopic location. Unilateral cryptorchidism may or may not adversely affect semen quality, whereas bilateral cryptorchidism results in sterility. Among unilateral cryptorchids the right testis is retained in the abdomen in about 90% of the animals.[23]

A higher incidence has been reported in intersexes, which is related to the polled characteristic. However, in Angora goats, cryptorchidism is not related to the polled intersex condition, which does not occur in this breed. Affected Angoras are genetic males.[23] The cryptorchid trait is recessive but controlled by a few pairs of genes.[24,25] Other possible etiologic factors of cryptorchidism include hormonal imbalances, mechanical obstruction such as fibrous adhesions, and focal infections along the course of descent, especially near the inguinal ring where many cryptorchid testes are located.[19] Normal abdominal temperature is about 5°C higher than scrotal temperature. Under the influence of abdominal temperature, the seminiferous tubules are not active and spermatogonia do not develop into spermatozoa. The Leydig cells are not affected and produce androgens; therefore, libido of cryptorchid animals is normal and physical features are masculine.[23] To prevent this condition, cryptorchid bucks should not be used for breeding and their sires and dams should also be culled. Even stricter selection would involve culling all offspring of known carriers.

Orchitis and Epididymitis

Bacterial orchitis and epididymitis are far less common in goats than in sheep. During the acute stage, orchitis usually presents with a typical clinical picture. The affected testes are hot, swollen, and painful. Affected bucks show systemic signs including pyrexia, depressed appetite, loss of libido, and inability to walk long distances. The testes usually swell rapidly and sometimes become twice their normal size within 24 hours after early signs are noticed. In chronic orchitis, there may be marked loss of testicular mobility within the scrotum due to extensive fibrous adhesions. The testes become indurated and fibrous as the changes result in atrophy. Coliform bacteria and *Pseudomonas* have been cultured from ejaculates of young bucks. Coliforms have caused both orchitis and epididymitis when injected into the testis.[26] *Actinobacillus seminis* was isolated from 4 of 40 Angora bucks examined in South Africa, but details related to clinical presentation were not given.[27]

Sperm Granulomas

Sperm granulomas are common causes of sterility in bucks. They are frequently associated with the polled condition but have been observed in horned bucks. This condition is believed to be heritable and recessive, with incomplete penetrance (estimated in one study as 0.55).[28] The normal head of the epididymis forms from the union of approximately 16 to 19 efferent ductules.[29] The direct cause of obstruction is believed to be one or more of these ductules that end blindly.[28] The ducts become distended with inspissated sperm until rupture occurs and sperm are released into the stroma of the epididymis. A severe inflammatory reaction with lymphocytes and giant cells occurs in response to the sperm cells and eventually a granuloma forms, which may calcify. Back pressure from granuloma formation eventually leads to degeneration and even mineralization of the testicular stroma.[28,30] Some animals are sterile because all of the efferent ducts are affected. Others may be initially fertile but become infertile if bilateral granulomas form. Normal libido persists. The firm granulomas in the head of the epididymis and reduced size of the testes will be palpable. Less commonly, granulomas form in the body or the tail of the epididymis. On ultrasonographic evaluation, appearance of sperm granulomas has been described as fluid-filled structures with a ring of echogenic tissue.[18] Ultrasonography is also useful for demonstrating testicular mineralization, the end stage of degeneration induced by back

pressure. Correction of the defect is not possible. The diagnosis can be confirmed by gross and histologic examination after castration or slaughter.

Scrotal Hernia

Scrotal and inguinal hernias have not been described in goats but have been observed by the authors in sheep and are presumed to be hereditary in that species. Clinical signs include distention of one side of the scrotum with a freely movable, fluctuant loop of intestine. If a hernia is identified and surgical correction is desired, the buck should be castrated bilaterally at the same time.

PENILE AND PREPUTIAL ABNORMALITIES

Hypospadias

Hypospadias and failure of preputial closure are diagnosed by inspection of the external genitalia and by catheterization of the urethra. The external urethral orifice opens on the ventral aspect of the penis (rather than at the tip) or in the perineum when hypospadias is present. Affected animals may be asymptomatic or may have urine scalds and infection of regional mucocutaneous surfaces. This condition usually is a part of the intersex condition commonly seen in goats.[23] Evaluation of the karyotype and gonadal histologic features may further characterize this disorder as an abnormality of sexual differentiation.

Posthitis

Posthitis, also termed "pizzle rot," is a chronic condition characterized by scabs and ulcers of the skin and mucosa of the prepuce and is caused by an interaction of a high-protein diet and *Corynebacterium renale*. Free ammonia scalds the preputial and penile mucosa. Wethers appear to be more susceptible than intact bucks. Pustules and ulcers on the prepuce and penis of bucks have been described as caused by a herpesvirus that is associated with vulvovaginitis in does.[31] The gross appearance varies with the extent of secondary bacterial infection. Histologically, findings of acidophilic intranuclear inclusion bodies and chromatin margination in epithelial cells adjacent to ulcers are considered highly suggestive of herpesvirus infection.[32] It is also reported that bucks that spread the infection to does during mating may remain free of clinical signs.[33]

Phimosis

Phimosis is suspected in bucks when the penis cannot be extruded, when there is decreased libido or inability to copulate when females return to service. The exact cause of the condition is not known. Adhesions of the penis in the region of the sigmoid flexure may be due to trauma from horn injuries. Phimosis in younger animals without evidence of trauma is considered to be a congenital condition that causes permanent unsoundness.[34] It must be differentiated from the normal inability of the prepubertal buck to extrude the penis owing to the presence of adhesions between the penis and prepuce.

Paraphimosis

Inability to withdraw the penis into the prepuce results in edema, swelling, and balanoposthitis. The prognosis is guarded and depends on the diligence of treatment and degree of trauma or necrosis present.[19] Treatment includes cleaning of the prolapsed penis with mild antiseptic solution and removal of necrotic tissue. Attempts should be made to get the penis back into the prepuce as soon as possible. Penile edema may be reduced by frequent hydrotherapy, use of diuretics, and application of ointments or Epsom salt packs. Treatment may be warranted only in valuable bucks. In most cases, culling of affected animals is recommended.

Urinary Calculosis

Urolithiasis is a common problem in castrated bucks (wethers) but also occurs occasionally in intact males. Various management factors, including deprivation of water and increased feeding of excessive minerals (particularly phosphate), contribute to urinary calculosis.[19] Urolithiasis is usually diagnosed on the basis of typical signs that begin with uneasiness, arched back, straining, and attempts to urinate accompanied by rapid side-to-side movement of the tail. In some bucks, small amounts of urine leak from the prepuce and, after evaporating, leave deposits of crystals on preputial hair. Later, the affected animals may kick at the belly, refuse food and water, prefer to lie down, become rather dull, and isolate themselves from the flock.

If the obstruction is at the base of the urethral process, urethrostomy can be performed by simply placing the patient on its rump and flexing its back to prolapse the penis through the preputial orifice. The penis is manually grasped and the urethral process is cut at the junction of the glans penis with scissors. If urination is normal following the operation, fertility is not usually impaired.

DISEASES TRANSMITTED BY SEMEN

Agents known to be transmitted in semen include foot and mouth disease virus, bluetongue virus, leptospiras, mycoplasmas, and toxoplasmas. Agents that are probably transmitted include *Brucella melitensis* and *Mycobacterium paratuberculosis*.[6] Most infectious agents can survive the semen freezing process. However, extended semen may contain less than the minimum infective dose. If bucks are kept in artificial insemination centers, they can be routinely screened for most of these diseases. Custom semen freezing centers have reported poor semen quality and freezability when semen is processed from bucks with severe CAE that have received phenylbutazone.[6] This might be because of effects of the virus on testicular function or decreasing body condition as the disease advances rather than because of a toxic effect of anti-inflammatory drugs.

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CHAPTER 67

Urogenital Surgery in Goats

A. M. HEATH, DAVID GARTRELL PUGH, and M. S. EDENS

The most common urogenital surgery that will be performed by most practitioners is castration of young bucks. However, as the goat is becoming a preferred pet animal in many households, knowledge of some common urogenital emergency procedures is becoming vital. Two of the most common reasons for veterinarians to see individual goats are for the relief of dystocia in the female and urolithiasis in the male. Both of these conditions often result in emergency surgery. The preparation of teaser animals for detecting females in estrus for artificial insemination programs is also discussed in this chapter.

MALE UROGENITAL SURGERY

Surgical Castration

The most common surgical procedure performed on goats is castration. Complications include excessive hemorrhage, evisceration, infection, rupture or tearing of the ureter, and tetanus.[1] Tetanus prophylaxis should include the use of toxoids and antitoxins. Evisceration appears more common in young goats, and possibly has some breed predisposition (pygmy[1]). Routine castration of kids is usually done the first week of life. However, if the kid is to be a pet, it is advisable to wait until at least 5 to 6 months of age in order to allow for maturation of the penis, urethra, and detachment of penile adhesions.[1]

Animals may be sedated, anesthetized, or restrained by an assistant. Young lambs or kids 2 to 4 days old are often surgically castrated without anesthesia. Light sedation of pet goats can be beneficial owing to their propensity for vocalization. Local anesthesia can be achieved by local infiltration of a 1% lidocaine hydrochloride solution in the scrotum and around the spermatic cord. Large, adult bucks should be sedated (xylazine hydrochloride, 0.05–0.3 mg/kg IM) as they may be very susceptible to shock associated with the stress and pain of castration.[1] The lower one third of the scrotum is removed by a scalpel blade to expose both testicles. The scrotal fascia is stripped away and testicle is identified and pulled by steady traction ventrally while the cremaster muscle and the remaining scrotal fascia are torn away from the spermatic cord. Completely tearing away the cremaster muscle and the scrotal fascia will allow more of the spermatic cord to be exposed to ensure it is broken above the pampiniform plexus. The cord is broken by applying constant downward pressure on the testicle with one hand to expose as much of the spermatic cord as possible and then breaking the cord as dorsally as possible by applying pressure on the cord with the other hand.[1] Breaking the cord above the pampiniform plexus will generally result in less hemorrhage than breaking the cord at or below the plexus.

In bucks older than 4 months of age and during the breeding season, it may be necessary to place an emasculator or transfixation ligature dorsal to the pampiniform plexus, in order to control hemorrhage. If hemorrhage appears to be clinically significant, the bleeding vessels should be ligated. If bleeding vessels cannot be identified, the scrotum can be packed with sterile gauze (soaked in antiseptic iodine or epinephrine) and the scrotum sutured closed. An alternative would be to close the scrotum with purse-string or a through-and-through suture pattern in order to reduce dead space within the scrotum. The day after surgery the scrotum should be reopened and the gauze removed. If the scrotum is packed, the animal should be placed on antibiotics for 1 to 4 days. On rare occasions when the testicles are pulled, the testicular artery may be avulsed from the aorta, which can result in fatal hemorrhage.[2] Animals need exercise after castration in order to reduce postoperative swelling.[1]

Elastrator Band Technique

The use of the elastrator band is the most commonly used technique by many producers on animals under 3 to 4 weeks of age. A heavy rubber band is placed around the neck of the scrotum. The entire scrotum is included within the bands.[1] Pulling the testicles as far ventrally as possible away from the abdominal wall before placing the bands will prevent trapping penile structures (sigmoid flexure) within the band. Elastrator band methods are considered inhumane by some if performed on animals over 1 to 3 weeks of age.[2] The scrotal sac and the trapped testicles undergo ischemic necrosis and slough within 2 weeks. It has an advantage of no open wounds or hemorrhage, but is associated with an increased risk of tetanus.[2] Occasionally the blood supply to one or both testicles will not be occluded, and the testicles will remain and continue to function.[2]

Burdizzo Emasculatome

Another method for castrating sheep and goats involves using the emasculatome. There are no open wounds and compared to elastrator bands there is a decreased risk of tetanus. Anesthesia is not required. The testicle is pushed to the ventralmost part of the scrotum and each cord is held tightly against the lateral aspect of the scrotum with one hand while the Burdizzo emasculatome instrument is applied twice to each cord.[1] Clamping each cord sepa-

rately increases the likelihood of interrupting the testicular blood supply and decreases the incidence of scrotal sloughing. The complications of this method include testicular survival, scrotal sloughing, scrotal swelling, and tetanus. Some consider this an inhumane method of castration in older animals.[1]

Hemicastration

In cases of unilateral testicular disease, the decision may be made to remove the involved testicle in order to prevent heat-induced testicular degeneration of a normal testicle that may occur as a result of inflammation in the diseased testicle. General anesthesia is recommended. The male is anesthetized, placed in right lateral recumbency, and the entire scrotum and the surrounding area are clipped and aseptically prepared. Starting near the base of the affected testicle and extending to near the apex, an elliptical incision is made through the skin and tunica dartos on the lateral aspect on the scrotum. The incision should not be extended into the normal hemiscrotum. The affected testicle and its associated tunics are bluntly dissected away from the scrotum. The vaginal tunics are excised in order to expose the testicle and the spermatic cord. The spermatic artery and vein are ligated with a transfixation suture above the pampiniform plexus, and the cremaster muscle is ligated at a point proximal to the vascular ligature. A separate ligature is then placed around the entire spermatic cord. The cord is clamped approximately 5 to 6 cm distal to the vasculature suture, and the cord transected. The remaining vaginal tunic is transected far enough distally to allow the tunics to be closed over the remaining cord with an inverting suture pattern. Excess skin should be trimmed so that no dead space is present. The tunica dartos muscle and the longitudinal skin incision are closed separately. If excess hemorrhage is expected, the scrotum may be bandaged following surgery. The bandage should be removed within 12 hours after surgery to minimize thermal damage to the remaining testicle. Routine postoperative antibiotics (penicillin or tetracycline) should be continued for 4–5 days. Nonsteroidal anti-inflammatory drugs may be indicated for control of pain, swelling, and other signs of inflammation.[1]

Teaser Preparation

Teaser bucks greatly facilitate estrus detection for artificial insemination and embryo transfer programs, particularly if used in conjunction with marking harnesses. A young, healthy, postpubertal male with good libido would be the best choice. The male should be incapable of intromission in order to minimize the spread of venereal disease or contamination of the vagina prior to artificial insemination. He should also be rendered infertile in order to guarantee no offspring are produced.

Vasectomy

A properly performed vasectomy will render the male incapable of fertilization, but will not prevent intromission. The animal is mildly sedated (xylazine 0.05 mg/kg IM) and the area proximal to the scrotum is infiltrated with lidocaine and prepared for aseptic surgery. The spermatic cord is identified and the overlying skin is incised. A combination of blunt and sharp dissection is used to carefully isolate the vas deferens from surrounding structures of the spermatic cord. Two ligatures are placed 4 cm apart on the vas deferens and a 2-cm section of the vas deferens is removed. The skin is closed with sutures, staples, or by a subcuticular pattern.[3] The procedure is repeated on the remaining vas deferens. Alternatively, the procedure can be performed through a single incision over the spermatic cord. The buck should not be used for estrus detection for 30 days.[1]

Epididymectomy

Like the vasectomy, an epididymectomy will not prevent intromission but will prevent emission of sperm. Bilateral caudal epididymectomy is a simple surgical procedure and easier to perform than a vasectomy.[4] The distal scrotum is aseptically prepared. A local anesthetic is infiltrated in the skin over the tail of the epididymis. The testis is forced distally in the scrotum until the tail of the epididymis is easily identified through the scrotal skin. A skin incision is made over the epididymal tail and continued through the common vaginal tunic until the tail of the epididymis is exposed. The tail of the epididymis is grasped with a pair of towel forceps, and dissected away from the testicle. The epididymis should be isolated and sutures placed proximal and distal to the tail of the epididymis. A portion of the epididymis is removed. In an alternative method, a pair of forceps are clamped across a loop of epididymis and this portion of the epididymis removed. The authors suggest the loop of epididymis be stored in a container with formalin labeled with the owner's name and the goat's identification. This can later be used as evidence that the surgery was performed correctly. If the epididymis is sutured and the procedure is performed under aseptic conditions, the scrotal skin may be sutured. In less than sterile conditions, the skin is left open to granulate. A minimum of 30 days of sexual rest should be enforced before use in a teasing program.[1,3]

Penile Translocation

A surgical procedure useful to prevent intromission is translocation of the penis to the left flank. A vasectomy or epididymectomy should also be performed in conjunction with this surgery in order to ensure sterility. The buck should be given a systemic antimicrobial 2 to 4 hours prior to surgery. With the buck standing, an area 1 cm cranial to the flank fold is marked.[1,4] The buck should then be either heavily sedated, anesthetized with injectable anesthetics, intubated and maintained on gas anesthesia, or given a lumbosacral epidural. The goat is placed in right lateral recumbency and the ventral abdomen and left flank are clipped and prepared for aseptic surgery.[4,5] A 4-cm circle of skin and cutaneus trunci muscle is excised 1 cm above the flank fold, just cranial to the mark made on the left flank. This area is covered with saline moistened gauze.[1] A 4-cm circumfer-

ential skin incision is made around the preputial orifice. A single skin suture is placed at the dorsal aspect of the preputial orifice.[4] The skin incision is extended longitudinally along the penile shaft, two thirds of the way to the scrotum.[1] The penis and nonhaired prepuce are freed of the subcutaneous tissue. A tunnel under the cutaneus trunci muscle is created by scissors or blunt dissection from the circular incision in the flank to the caudalmost aspect of the longitudinal incision over the penis. A towel forceps is now passed through the tunnel starting at the circular incision and exiting near the penis. The penis, with a sterile glove covering the preputial orifice to prevent contamination, is then repositioned through the tunnel by using the towel forceps to grasp the skin of the preputial orifice. The penis is then pulled dorsally so that it is at a 45-degree angle to the long axis of the body and the orifice is positioned within the circumferential skin incision.[4] Care should be taken to prevent twisting the penis or prepuce. The suture at the orifice should be used to align the dorsal aspect of the preputial orifice with the dorsalmost point in the circular incision. The subcutaneous tissue of the preputial orifice is sutured to the cutaneus trunci muscle. One absorbable simple interrupted suture is place at each quadrant, and then additional sutures are placed within each quadrant to completely oppose the tissue. The skin of the preputial orifice is then sutured to the skin of the flank incision in a similar manner using nonabsorbable suture. The subcutaneous tissue along the ventral longitudinal incision into the prepuce is closed. The skin can be closed using either simple interrupted sutures or a continuous pattern. The cranial aspect of the incision (where the preputial orifice was located) is left open to allow for drainage.[1,4,5] Tetanus prophylaxis (tetanus toxoid or tetanus antitoxin) and fly control should be instituted. A bilateral epididymectomy or vasectomy is performed, and antibiotics continued for 5 to 7 days (Procaine penicillin 22,000 IU/kg twice a day). The sutures are removed in 14 days. The male will be ready for use in a teasing program within 1 month.

Surgical Treatment for Urolithiasis

Clinical signs of urolithiasis include straining to urinate, continuous dribbling of urine, colic, vocalization, and flagging of the tail. Small mineralized crystals can sometimes be found on the preputial hairs of the goat. These goats are often presented with numerous acid-base imbalances and electrolyte abnormalities. Increased blood urea nitrogen (BUN) and creatinine concentrations are sometimes noted. These abnormalities should be addressed in the course of preparation for surgical intervention.

Several surgical techniques are used to manage cases of urolithiasis. Whenever treating urolithiasis, the clinician should evaluate the diet of the affected animal, analyze any stones encountered, and include medical therapy where appropriate. Risk factors that have been associated with urolithiasis include high concentrate rations, low roughage rations, a low Ca:P ratio, high magnesium diets, and alkaline urine. High-protein diets may also contribute to the formation of mucoproteins that tend to coalesce with mineral crystals. In general, the treatment of most cases of urolithiasis will include some form of surgical intervention in combination with dietary management. Urine acidifiers (ammonium chloride, 2–4 g daily per os) are used to try to maintain the urine at a pH of between 6 and 6.5.[6,7]

Amputation of the Urethral Process

The first step that is taken when a goat is presented with the inability to urinate is to amputate the urethral process. The urethral process is identified as a slender appendage at the distal end of the penis and is simply cut off with a pair of surgical scissors. Although this will often result in immediate relief of the blockage, the problem may recur with subsequent calculi production blocking the flow of urine at other sites. Future stenosis of the excision site is also a possibility. The goat should be monitored closely for reobstruction and the diet appropriately modified.

Perineal Urethrotomy

Perineal urethrotomy is a commonly performed procedure in male goats, but surgical failure, poor long-term survival rates (strictures), and loss of reproductive function decrease the usefulness of this procedure.[6,8,9] Either general anesthesia or epidural anesthesia may be employed for the procedure. The perineal area is scrubbed and a 10-cm midline skin incision is made below the ischial arch. Careful sharp dissection is used to expose the retractor penis muscles, the bulbospongiosus muscle, and the underlying penis. An incision is made through the median raphe of the bulbospongiosus muscle and into the underlying urethra. Palpating the distinct urethral groove on the ventral surface of the penis identifies the urethra. The urethra can be irrigated in both an anterograde and a retrograde fashion to help dislodge any calculi. This should be done with extreme caution so as not to rupture the urethra that may already be compromised. A Foley catheter can be placed into the bladder through the incision to allow easy flow of urine and to divert urine flow away from the surgical wound. The incision can be allowed to heal by granulation. Alternatively, a more ventral approach can be made and the urethral mucosa and the tunica albuginea may be sutured to the skin to create a urethrostomy.[6,10–12] The remaining incision dorsal and ventral to the urethrostomy site should be closed. The Foley catheter is maintained for 3 to 4 days in either procedure.[10] Antibiotics may be used at the surgeon's discretion. A cystotomy should be considered if there is the potential for substantial calculi to be retained in the bladder.

Penile Amputation

Amputation of the penis provides a simple approach to relieving urethral obstructions. However, this procedure may not be as cosmetically appealing and strictures may occur after surgery. It should, however, be considered as the treatment of choice for long-term survival when the urethra has already ruptured and there is significant damage to the distal portion of the penis and surrounding tissues due to urine accumulation. The surgery can be accomplished under either general or epidural anesthesia. A midline incision is made in the perineum dorsal to the

sigmoid flexure at the point where the perineum turns ventrocranially. Careful sharp dissection is made to expose the penis. The distal sigmoid flexure is identified and pulled to the incision site. If there has been significant urine damage to the preputial tissues due to urine leakage, the entire distal penis can often be extracted from the wound with moderate pressure. The penis is avulsed from its preputial attachment. A point on the penis 4 to 6 cm distal to the dorsal edge of the skin incision is chosen for the amputation site. The dorsal penile vessels are ligated dorsal to this point and the retractor penis muscles are ligated and transected as far proximally as possible. If the distal penis is not removed, the dorsal penile vessels should be reflected off the penis and left intact. The penile stump is sutured to the skin by placing a suture near the dorsal edge of one side of the incision, going through the body of the penis, exiting through to skin on the opposite side and returning the suture in the same manner to the starting side to form a horizontal mattress suture. The skin incision is closed above and below the penis. The urethra is split to spatulate the opening in order to prevent stricture formation. The corpus cavernosum (CCP) is sutured closed to prevent bleeding that will occur when intact bucks are sexually stimulated. A wedge-shaped piece of tunica albuginea is removed from each side of the penis in order to facilitate closure. Castration at the same time as the penile amputation is prudent.[13]

Cystotomy or Tube Cystotomy

Cystotomy provides the best long-term survival and the return to normal breeding of bucks with urolithiasis because the urethra is not surgically invaded. It should also be considered as an option for the therapy of pet animals to ensure the health of the urethra and ultimately a more cosmetically appealing cure. The buck is placed under general anesthesia and placed in dorsal recumbency. A right paramedian incision is made (2.0 to 3.0 cm off midline) extending cranially 8.0 cm from the teats.[6,9–12] The urinary bladder is retracted out of the abdomen and stay sutures are placed at either end of a proposed cystotomy site (dorsal aspect of the bladder) in order to allow for stabilization of the bladder. The bladder is incised, lavaged, and massaged in order to remove all calculi.[6] Mild normograde urethral flushing with an isotonic solution or combining normograde and retrograde flushing can help remove urethral calculi.[1,6] If normal flow through the urethra can be established, the bladder should be closed in an inverting pattern avoiding penetration into the lumen of the bladder.

If any doubt remains about the function of the urethra, a Foley or mushroom (16–24 F) catheter is placed into the bladder. This procedure should allow for a decrease in urethral inflammation and urethral spasms and will subsequently allow normal urine flow through the urethra.[9] A small skin incision is made lateral to the abdominal incision and the catheter is then tunneled subcutaneously prior to penetrating the abdomen lateral to and separate from the original paramedian incision. This is placed in the same manner as one would place a chest drainage tube. Layers of omentum are pleated over the end of the catheter. After the Foley catheter is positioned in the abdominal wall, a purse-string suture is placed in the ventrolateral bladder wall. A stab incision is made in the middle of the purse string, the balloon end of the Foley catheter threaded into the bladder, and the purse string suture is tightened around the catheter and the bulb is inflated.[6,9] The catheter is pulled outward so that the bladder is positioned near the lateral body wall. The catheter is secured to the skin on the lateral body wall and the original abdominal incision closed. A valve should be placed over the end of the catheter to help in the prevention of an ascending infection. The finger of a latex glove taped over the end of the catheter will provide this valve effect.[6,9,11,12]

Bandages and Elizabethan collars should be used to prevent the goat from damaging the tube.[6] Animals should be closely monitored for signs of depression, anorexia, or abdominal pain.[6] A clamp is placed over the catheter 4 days after surgery to impede urine flow. This will allow the clinician to access the goat's ability to urinate normally. If the goat is still unable to urinate normally, the catheter clamp should be released and the goat evaluated again the next day. Goats should be able to urinate normally for 2 days before deflating and removing the catheter. The Foley catheter should not be removed prior to day 7 postoperatively.[9] The bladder defect is allowed to heal spontaneously.

Bladder Marsupialization

When other surgical corrections have failed to provide a normal urine flow, a salvage technique such as bladder marsupialization may be considered.[6,11,13] Complications from this procedure include urinary incontinence, urine scald, and cystitis.

An 8- to 12-cm paramedian incision is made lateral to the prepuce. The bladder apex is exteriorized, stay sutures placed 4 to 5 cm apart, and a cystotomy incision made between the sutures. A second abdominal incision is made on the opposite side of the prepuce in a location far enough cranial to prevent urine scalding. The bladder apex should be positioned with minimal tension into the second abdominal incision by the stay sutures. The bladder is tacked to the abdominal wall with four evenly spaced sutures. The serosal and muscular layers are sutured to the abdominal fascia to form a circular stoma. The bladder mucosa is then sutured to the skin. The original abdominal incision is closed in a routine fashion.[11,13] The surgical stoma should be large enough to allow urine flow, but not too large as to allow bladder eversion. Short-term complications of marsupialization are bladder prolapse and cystitis, and long-term complications are closure of the stomal site.[6,13]

FEMALE UROGENITAL SURGERY

Cesarean Section

Often due to the small size of the patient, the clinician may find it more difficult to vaginally manipulate the fetuses in goats than in other domestic animals. Therefore, cesarean sections are recommended when vaginal delivery cannot be accomplished and the animal's value will allow this procedure.[1] Mild sedation with acepro-

mazine, in conjunction with leg restraints and an inverted-L block with 10 to 20 ml 2% lidocaine provides good anesthesia. Deeper sedation is attainable with a mixture of Telazol (6.6 mg/kg IV) and ketamine (6.6 mg/kg IV). Although other approaches are possible, a recumbent, left flank approach has the advantage of easy restraint.[1] The front and rear legs are restrained in extension with soft cotton ropes. A rolled towel is placed under the spine to tilt the doe to a 30-degree angle toward the surgeon. The paralumbar fossa is clipped and prepped aseptically. A skin incision is made near the center of the left paralumbar fossa. Depending on the size of the doe, the incision should start 5 cm ventral to the lateral processes of the lumbar vertebrae and continue in a slight ventrocranial direction for approximately 15 cm.[1]

The external and internal abdominal oblique muscles can be excised in the same plane as the skin incision. An incision is carefully made through the transverse abdominal muscle and the peritoneum. This incision can be extended by inserting fingers into the incision and pulling both dorsally and ventrally to separate the muscle along the direction of its fibers.[1]

The uterus is located and partially exteriorized. Sterile pads are packed around the exposed uterus to decrease abdominal contamination. The uterus should be incised longitudinally over the greater curvature of the horn in a relatively avascular area.[1] The incision should be long enough to allow easy removal of the fetus without risking uterine tears. Multiple incisions may be needed in the case of twins or triplets. After removal of the fetuses, the incision should be closed with an inverting suture pattern (e.g., Utrecht pattern). If a good closure is not obtained, the entire incision can be oversewn with a second inverting closure. The transverse abdominal wall is closed along with the peritoneum with a simple continuous pattern. The internal and external abdominal obliques may be closed together and the skin closed in a routine fashion.

Ovariectomy

Bilateral ovariectomy is performed to produce females for use in semen collection and libido testing of males. These ovariectomized females should be given estradiol (100 mg) 24 to 48 hours prior to use.[1,14] Other indications are for the treatment of ovarian tumors and for sterilizing pet animals. A flank or a midline approach is acceptable to perform ovariectomies. The animal should be heavily sedated and the incision site blocked with a local anesthetic or general anesthesia may be used. Animals should be given antibiotics up to 4 hours preoperatively and the clinician should adhere to aseptic technique. The abdomen should be entered as far caudally as possible and the ovaries are located. The ovarian pedicle should be identified and the ovary gently exteriorized. A crushing forceps is placed on the ovarian pedicle and a transfixation ligature is placed around the pedicle. The ovarian pedicle is transected. The hemostat is released and the pedicle examined for hemorrhage. The abdominal incision is closed in a routine manner. The procedure may also be performed through a ventral midline incision. This may be more appropriate for larger goats and primiparous goats.

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CHAPTER 68

Techniques for Artificial Insemination of Goats

LOU NUTI

Artificial insemination (AI) involves placement of semen from a male into the reproductive tract of a female by mechanical means rather than by natural mating. A thorough understanding of the various components of an AI program is very important to goat breeders if the herd is to be handled effectively and efficiently. This chapter is designed to provide readers with a foundation to build a sound AI program.

ADVANTAGES OF ARTIFICIAL INSEMINATION

The contribution of AI to the genetic makeup of highly productive strains and breeds of cattle during the past 40 years has made AI a desirable tool for genetic improvement in other species. AI offers goat breeders the potential for use of genetically superior sires. It enables the small herd owner to obtain breeding services at a reasonable cost. By means of AI, breeders are able to identify superior animals that possess desirable traits. The mating of outstanding sires and dams, even miles apart, may be effectively performed by AI using frozen semen. Such matings have the potential to create desirable new lines, reduce inbreeding, and reduce spread of disease by minimizing animal movement. When used correctly, AI has a number of benefits, including maximal use of outstanding sires, increased herd uniformity, elimination of bucks on the farm, relatively inexpensive semen costs, decreased potential for venereally transmitted diseases, and improved herd management.

DISADVANTAGES OF ARTIFICIAL INSEMINATION

The disadvantages of using AI should also be understood and carefully examined before the herd manager decides on this program for a herd. These include initial costs for AI equipment and nitrogen refrigerator, increased labor for heat detection and insemination, lack of standardized procedures for packaging and quality control for goat semen, lack of suitable sire proofs for production traits, requirement for specialized training because of anatomic constraints associated with the size of the goat, and potential for spread of less desirable traits.

SIGNS AND DURATION OF ESTRUS

The signs of estrus are primarily changes in behavior, one or more of which may be exhibited by most does. It is important to become familiar with and to record these signs for individual does, including time of onset, duration, and intensity. These signs are valuable pieces of information that can be used to determine optimal time for natural mating or AI. Signs include increased frequency of bleating, frequent urination, obvious restlessness or an increase in doe interaction, tail flagging, increased curiosity and attentiveness to the herd handler, noticeable change in milk volume in lactating animals, exhibition of male behavior by does (mounting, snorting), receptivity to mounting (standing heat), and anatomic changes including swelling of the vulva, discharge of vaginal mucus, and hyperemia of the vulva.

ESTRUS DETECTION AND ARTIFICIAL INSEMINATION

One of the most important, if not the most important, aspects of goat breeding when hand mating or AI is used is proper detection of estrus. Because the noticeable signs of estrus are primarily changes in behavior, familiarity with the normal behavior of individual does is required. These behavioral changes along with anatomic changes are the best tools to use to time mating either by natural service or by AI.

When bucks are available, does actively seek males when they are in estrus. The odor of bucks seems to have a stimulatory effect on does. The most dependable sign of estrus is the doe's response to a buck. A doe will remain immobile for breeding only during this period of standing heat, which lasts from 12 to 48 hours. Successful AI requires that human judgment be substituted for a buck's instinct and libido. If a person is less diligent or conscientious in the task of detecting heat than the buck, then some does in estrus will be missed and not mated.

Herd management conditions determine the exact procedures to be followed in observing for signs of heat. Does should be observed at least twice a day when the animals are free from distractions. Lactating animals can be observed shortly after each milking. Open, nonlactating does in loose housing or at pasture must be observed carefully. These animals may have to be confined twice daily. Choosing the right time of day (early morning and late night when it is cool) and location (shade, water source, near buck pens) will help tremendously.

Nothing helps detection of does in heat as much as accurate animal identification such as tags with large numbers or clear brands, and a record-keeping system

that indicates when particular animals require observation. Individual doe cards and large calendars are often used to keep simple "heat due" dates.

IMPORTANCE OF TIMING IN ARTIFICIAL INSEMINATION

It must be understood that a doe is fertile only when an ovum is present that can be fertilized. Likewise, it must be understood that ova are viable for only a short time after being ovulated unless they are fertilized. Does do not ovulate until late estrus or shortly after the end of standing estrus. Therefore, for optimal results does should be inseminated during the latter half of standing heat or shortly after the end of standing heat.

The accuracy of recognizing the signs of standing heat is at best fair and is generally reflected in the success of AI. Quite often, goat breeders follow the AM:PM rule used in breeding cattle; animals first noticed in heat in the morning are bred in the afternoon and animals first noticed in heat in the afternoon are bred the following morning. Although this system works moderately well, it is no substitute for exact information regarding individual estrous behavior and length. The author's personal preference is to inseminate at least 12 hours after the first observation of heat, with preference for late rather than early insemination, provided that animals are checked regularly at least twice a day at 12-hour intervals. This preference is based on information available regarding the time of ovulation, duration of sperm transport in the female reproductive tract, and time of survival of male and female gametes.

AIDS FOR SUCCESSFUL HEAT DETECTION

A number of aids have been developed to assist with the task of detecting does in estrus. These include the use of a "buck rag," which is a cloth that has been wiped over the scent glands of a male in rut, stored in a closed jar, and warmed before opening in the presence of the does; a sturdy and secure buck pen with a breeding window in an area where does are brought in for heat detection; the use of sexually altered (by penile deviation or vasectomy) males fitted with a marking harness; use of wethers or intact females treated with testosterone; and the use of intersex goats or hermaphrodites.

HANDLING OF SEMEN

The procedures used for handling semen, beginning from the time it is shipped from the supplier until it has been deposited in the doe, have a tremendous influence on fertility. Low temperature enables semen to be stored for extended periods of time once it has been frozen, but the fact that semen remains frozen does not ensure that fertility has been maintained. Damage can be expected any time the temperature of frozen semen rises above –130°C, and the rate at which semen deteriorates increases as the temperature rises. Although liquid nitrogen has a temperature of –196°C, the potential for damage due to elevated temperatures is present when semen is transferred between tanks and when semen is elevated into the neck of a tank so that some can be removed for thawing. A current inventory of the location of semen within a storage tank and use of procedures to minimize both the extent of warming and length of time semen is exposed to elevated temperatures during routine handling are essential to maintenance of high fertility. Thawing techniques differ between ampules and straws, and among various types of straws. Research on thawing semen in ampules or straws has concluded that semen in ampules should be thawed in ice water while semen in straws should be thawed in water at about 35°C.

Although most researchers now recommend that semen in straws be thawed in water at about 35°C, controversy exists as to whether the semen should remain in the thaw bath until its temperature has reached the temperature of the water or whether thawing should be timed so that the semen temperature does not rise above 5°C. Recent work on cattle semen in a large trial concluded that thawing need not be timed to prevent semen temperature from rising above 5°C, and that fertility may actually be improved by allowing semen temperature to rise to 35°C.

Another controversial issue, with respect to semen in straws, is the importance of using the semen immediately upon thawing. Although most cattle AI organizations recommend that thawed semen be used as soon as possible, they have expressed little concern at allowing semen to remain in the thawing bath for several minutes. In goats, however, it is recommended that semen be thawed for 30 to 60 seconds at 35°C because of the likelihood that the semen will spend several minutes in the doe's tract while the inseminator tries to penetrate the cervix.

NUMBER OF SPERM PER INSEMINATE

The number of sperm per inseminate is an important factor influencing the fertility of frozen semen. The optimal number of sperm per inseminate depends on the fertility of the sire, inseminator competence, and the timing of insemination relative to ovulation. Goat semen processors are best qualified to determine the optimal numbers of sperm cells based on client feedback. Few field trials titrating sperm numbers against conception rates have been conducted for goats. As a result, most goat semen processors package goat semen in concentrations that yield 50 to 100 million progressively motile sperm cells after freezing and thawing. With suitable processing and careful handling and thawing, at least 50% of the cells survive and are progressively motile. Thus, initial prefreeze concentrations should be between 200 and 400 million per milliliter of extended semen. Semen from certain sires survives freezing, storage, and thawing better than semen from others, for unknown reasons. Thus, knowledge of individual freezing ability of semen is essential for deciding how many sperm are necessary for each breeding unit.

Another important consideration with respect to sperm numbers has to do with the site of semen deposition. Laparoscopic insemination directly into the uterine horns requires fewer sperm than does transcervical insemination into the uterine body. Greater numbers of sperm are required for either cervical or vaginal

TABLE **68-1**

Minimum Safe Numbers* of Motile Spermatozoa for Insemination at Different Sites

	TYPE OF SEMEN		
Site	***Fresh***	***Liquid-Stored***	***Frozen-Thawed***
Vaginal insemination	300	Not effective	Not effective
Cervical insemination	100	150	180
Intrauterine insemination (total in two horns)			
Via cervix	60	60	60
Laparoscopically	20	20	20

*In millions.
Data from Evans G, Maxwell WMC: *Salamon's artificial insemination of sheep and goats.* Sydney: Butterworths Publishers, 1987.

insemination. Whatever the site of insemination, the number of motile spermatozoa in the inseminate affects fertility. Generally, less fresh extended semen will be needed than frozen-thawed semen. A recommended safe limit for the number of motile spermatozoa is shown in Table 68-1.

SITE OF SEMEN DEPOSITION

The site of sperm deposition within the reproductive tract of the doe has a marked effect on pregnancy rates. Semen should be deposited in the body of the uterus, although cranial cervical deposition has yielded satisfactory results (Fig. 68-1). In cattle, the reproductive tract can be palpated, but experienced inseminators deposit dye correctly into extirpated reproductive tracts only 24% of the time. Thus, it is likely that many failures in goat AI are the result of misplaced semen. In the author's experience, attempts to pass an AI gun have resulted in placement of the tip of the gun in one of the uterine horns or puncture of the cervix and placement of the tip of the gun in the abdominal cavity. Gauging the depth of AI gun penetration by marking the gun as well as developing the proper "feel" when applying pressure to pass the AI gun past the cervical rings should improve AI success rates.

Laparoscopic uterine insemination procedures have the advantage of depositing semen directly into the lumen of the uterine horns at a point closer to the site of fertilization than transcervical insemination. This also eliminates one of the primary barriers to semen deposition, namely, the cervix. Two stab incisions are made in the ventral abdominal wall approximately 2 cm on either side of the midline about 10 cm cranial to the udder. One is for insertion of the trocar-cannula (7 mm), through which the laparoscope is introduced. The second incision is for the secondary trocar-cannula through which the inseminating instrument (5 mm) is introduced. Several types of inseminating instruments have been developed. The inseminating gun with aspic needles* is a very useful and precise instrument. The needle is sharp and long enough to go through the uterine wall so that semen can be deposited within the uterine lumen (Fig. 68-2). If difficulty is encountered during injection or if swelling of the uterine wall occurs, the semen is not being injected into the uterine lumen. To ensure correct semen deposition, the inseminating needle must be perpendicular to the uterine horn and the needle must be introduced completely through the uterine wall. Alternatively, a Pasteur pipette can be used for this purpose.

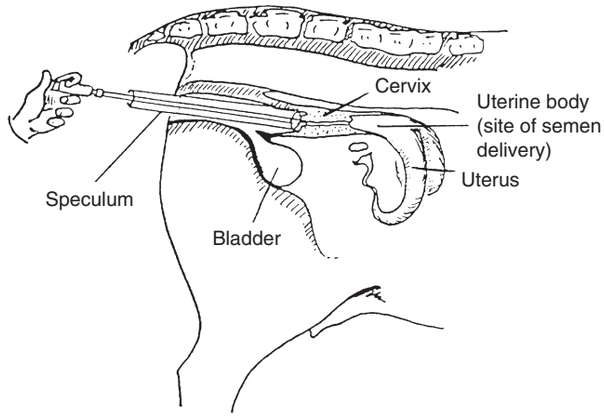

Fig. 68-1 Optimal site of semen deposition.

EXPULSION OF SEMEN

Because most ampules or straws contain no more than the minimum number of sperm needed for maximum fertility, care must be taken to prevent loss of sperm in straws, ampules, or insemination equipment. It has been shown that rapid expulsion (2–3 seconds or less) of semen from the catheter that was used to aspirate semen from ampules together with the use of high-viscosity extenders (e.g., 50% egg yolk/citrate) resulted in 27% semen residue remaining in the rod, which translates to loss. Although the inseminator has little control over the extender used, he or she can increase the number of sperm delivered significantly by expelling the semen over a 5- to 7-second interval. Losses are not as pronounced when straws are

*IMV International Corp, Minneapolis, MN.

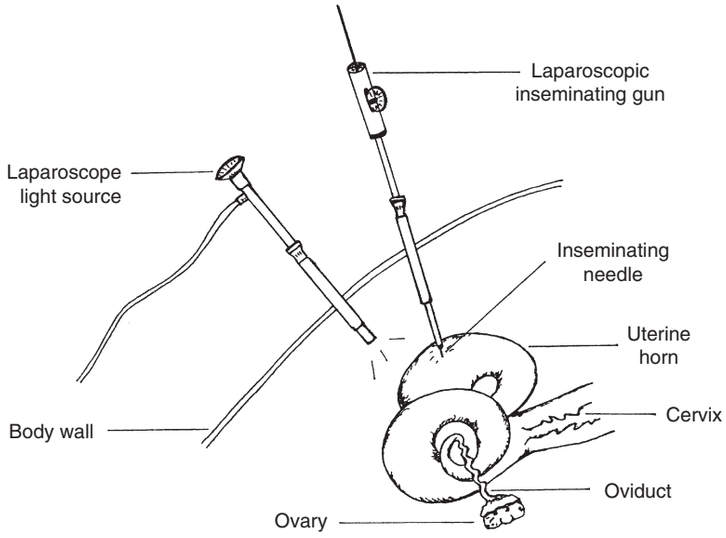

Fig. 68-2 Laparoscopic artificial insemination of goats.

used because semen is expelled directly from the straw by means of a metal plunger that forces a solid plug through the straw. Nonetheless, several seconds should be allowed when expelling semen from straws, because rapid expulsion may increase the chance for leakage of semen within the insemination device due to straw damage or improper seating of the straw.

INSEMINATOR COMPETENCE AND TRAINING

Inseminator errors in handling and placement of semen within the reproductive tract of does and the importance of proper timing of insemination have been discussed. It is suspected that these errors are the most common cause of low fertility when AI programs are unsuccessful in herds in which does are free of disease and have normal reproductive cycles. It should be clear that although frozen semen may be of excellent quality when it leaves a buck stud or AI facility, fertility will be poor if semen is improperly handled or deposited in the wrong place at the wrong time.

Experience per se does not ensure competence. Differences in success among "experienced" cattle inseminators, even among those closely supervised, have been reported. One would suspect that differences in conception rates may be even greater with owner-inseminators, probably because close supervision is generally lacking and proper techniques are forgotten and skills are lost during long nonbreeding periods. Retraining prior to the breeding season should be considered by such individuals. When large numbers of animals are to be inseminated in a short period of time, such as in an estrus synchronization program, inseminators should make certain that they are sufficiently conditioned against fatigue. If AI training workshops are not available, inseminators could try to spend time with someone who has had a consistently high success rate and watch closely for the step-by-step procedure used.

ARTIFICIAL INSEMINATION EQUIPMENT BOX

This box should be large enough to accommodate all the AI equipment. Insemination boxes can be acquired from an AI equipment supplier. These boxes are usually of a durable material or metal. The box should have a lid to keep the equipment clean and in order. The AI box generally contains an insulated thaw box, insemination gun (for 0.25- or 0.5-ml straws), sheaths, lubricating jelly, speculum, light source, thermometer, straw cutter, tweezers, paper towels, and a speculum brush. Anything that comes into contact with the internal reproductive tract of the doe should be sterile. Thus, special precautions should be taken in cleaning, handling, and storing the speculum, AI gun, light, and sheaths. The sheaths are usually prepacked in sterile bags. Once the sheath is removed from the bag, care must be exercised in handling to avoid possible contamination. Specula should be soaked immediately after use in warm, soapy water until they can be thoroughly washed in hot soapy water, using a test tube brush. Specula are then rinsed thoroughly and sterilized in boiling water, alcohol, or chlorhexidine disinfectant or by baking in a hot oven. Once cleaned, rinsed, and dried, each speculum is wrapped in a clean paper towel until its next use. All other equipment should be cleaned in a like manner. The insemination gun should be cleaned by taking the plunger out of the barrel and wiping each part thoroughly. All AI equipment should be stored in a dust-free container until use.

RECORD KEEPING FOR ARTIFICIAL INSEMINATION

A system for recording complete and accurate insemination data is an integral part of a successful AI program. The more detailed the observation of each insemination attempted, the more likely important clues will be noted that will improve future attempts. A 3-by-5–inch index

card kept in a recipe file box works well. Information usually noted includes the date of breeding; the number of straws used; the date of last service or AI; the name and number of the doe; the name and address of the doe's owner; the complete name and registration number of the sire; the date of semen collection and the processor; the appearance of the cervix; the quantity, appearance, and consistency of mucus at time of AI; the depth of transcervical insemination and degree of difficulty; the interval from thaw to insertion of semen into the doe; the time required for insemination; the time from first detection of heat to AI; the length of estrus after AI; and record of the semen processor's evaluation of the semen unit used.

REBREEDING FOLLOWING ARTIFICIAL INSEMINATION

If a doe is still in standing heat 12 to 24 hours after insemination, a decision must be made about whether to reinseminate. Most processors recommend a second insemination, but proper trials have not been conducted to justify the added cost. Until credible evidence is available, the decision must rest with the owner of the female. The cost and availability of semen and labor must be weighed against the likelihood of enhancing conception.

If the female appears to be in heat again 3 weeks subsequent to insemination, she should be examined with a vaginal speculum and an AI light. The cervical opening is located and inspected for evidence of a grayish mucus plug. If this plug is present, the doe is most likely pregnant. If the plug is disturbed, the doe may abort. Therefore, it is not recommended that AI be repeated on what appears to be a subsequent heat. If there is doubt that the doe is pregnant and rebreeding is desired, natural service is recommended.

TRANSCERVICAL ARTIFICIAL INSEMINATION

If a clean, comfortable site has been selected ahead of time, breedings have been planned, and insemination equipment has been kept in a clean, dust-proof container, then insemination of the doe should require only a few minutes. Tools and supplies should be assembled within easy reach of where the doe will be inseminated. Two clean specula (in case one should be dropped and broken), a package of disposable sheaths, lubricant, paper towels, tweezers, straw cutter or scissors, and inseminating gun should be laid out. The thaw container should be filled with water at 35° to 37.8°C. A thermometer should be used to make sure the water stays at this temperature. Some hotter water should be kept nearby and added if the water temperature falls below 35°C before the straw of semen is thawed.

A bucket of warm soapy water and a bucket of clear water should be prepared. The doe is restrained and the perineal area washed thoroughly with soapy water, rinsed, and dried with a paper towel. Next, the inseminator's hands are washed and dried. It is helpful to have an assistant restrain the doe and hold the tail to one side. The speculum is lubricated and inserted into the doe's vagina. If resistance is encountered, the speculum should be directed first dorsally and then slightly ventrally to pass over the ischial arch. After placing the breeding lamp inside the speculum, the entire speculum is rotated 180 degrees. The cervical opening should be located somewhere between the 5 and 7 o'clock positions. Once the cervix has been located, pressure is placed on the speculum to "lock" the cervix into the lumen of the speculum. If the doe is healthy and in standing heat, the cervix should be bright red and the external os relaxed, giving the appearance of a small black dot. If the cervix and cervical mucus appear typical of mid- to late heat, the AI procedure can be continued. If the mucus is thin and watery and the cervix is closed, the speculum should be removed and the doe checked again 6 to 12 hours later.

The assistant holds the tail and speculum in the doe while the inseminator proceeds. The semen storage tank is opened and the canister containing semen from the sire of choice is located. That canister is elevated up to the frost line in the neck of the tank. The desired cane is lifted out just far enough for the practitioner to reach into the goblet with the straw tweezers and remove one straw of semen. The identification on the straw is quickly checked and it is noted whether the straw is plugged at both ends. If it is plugged on both ends, the straw is placed in 35°C water for a minimum of 30 seconds. The straw can be left in the water bath for up to 4 to 5 minutes. If a straw is being used that is not plugged at both ends, a finger must be held over the unplugged end while the semen is thawing, as water is spermicidal. While the straw is thawing, the practitioner or assistant warms the insemination gun by rubbing it in a clean paper towel to create friction. This should be done immediately before loading the gun with the straw of semen. Warming the gun is a very important step, especially if the ambient temperature is low. The straw is removed from the thaw box and dried thoroughly with a paper towel, and the identification on the straw is rechecked. If an error has been made, refreezing the thawed straw should not be attempted. If the thawed semen cannot be used in the next few moments, it should be discarded. One end of the straw is plugged with white, cotton-like substance about 13 mm long. The opposite end is placed in the straw cutter and cut off. The cut end of the straw is placed into the open (nontapered) end of the sterile sheath. The warmed insemination gun is slid into the sheath with the plunger drawn out about 200 mm. Care should be taken to check that the cut end of the straw is seated squarely into the sheath adapter or the base of the tapered end of the sheath. The plunger is pushed gently until the air is expelled. The sheath is locked to the gun and kept warm but not hot.

When the cervix is located, the breeding gun is inserted through the speculum into the opening of the cervix. Then, using moderate pressure and a rotating motion, the breeding gun is manipulated through the rings of the cervix. A slight amount of forward progress should be felt each time one of the cervical rings is passed. Before proceeding too far through the cervix, a second sheath (one that has been marked 38 mm from the end) is inserted into the speculum until it touches the outside of the cervix. This sheath is used as a "yardstick" to

measure the actual depth of penetration that has been achieved. In no instance should penetration be greater than 38 mm to avoid perforation of the uterine wall or entry into one of the uterine horns. If a depth of at least 32 mm has been achieved, the semen should be deposited. If this depth has not been reached, the sheath can be rotated with forward pressure, but attempts to pass the insemination gun should not be prolonged. Continued efforts for more than 10 minutes are counterproductive. If the cervix has been penetrated at least 13 mm, there is a good chance that the sperm can negotiate the remainder of the cervical canal. If optimal penetration has been achieved, the insemination gun is slightly withdrawn so that the tip of the sheath is not pushed tightly against any tissue. The plunger of the breeding gun is then depressed very slowly and gently to deposit half the semen (about 50 mm on the plunger). The gun is then withdrawn to the midpoint of the cervix and the remaining semen slowly deposited. The inseminator removes the equipment from the doe by withdrawing the speculum about 50 mm and then gently and slowly withdrawing the speculum and insemination gun simultaneously.

When the insemination gun is removed and semen is seen between the sheath and the straw in any quantity, it is likely that "blow back" has occurred and instead of the semen entering the cervix, it flowed back into the sheath. If there is concern that insufficient semen was deposited, another straw may be thawed and the procedure repeated after ensuring that the straw is seated securely in the tapered end of the sheath. After insemination is complete, the doe is allowed to stand and relax for a few minutes while notes concerning the insemination are completed.

LAPAROSCOPIC ARTIFICIAL INSEMINATION

Feed and water should be withheld from does for at least 12 hours prior to laparoscopic AI. Sterile insemination equipment should be used and needles should be rinsed with sterile physiologic saline before use. Insemination equipment that comes into contact with semen should be kept at 30° C. All equipment should be checked for proper function, including the laparoscope light source. The trocar-cannula assemblies and body of the laparoscope should be placed in an appropriate sterilizing solution between inseminations. Does are prepared for insemination by light sedation. Does are placed in dorsal recumbency in a suitable cradle and the abdomen cranial to the udder is shaved and scrubbed. Local anesthetic is injected subcutaneously at two sites 5 to 7 cm cranial to the udder and 3 to 4 cm lateral to the ventral midline.

The cradle is raised to position the doe head down at an angle of 40 degrees or more from horizontal. Each trocar-cannula is inserted into the peritoneal cavity at the anesthetized sites. The laparoscope is inserted through the appropriate cannula. The peritoneal cavity is inflated with a small amount of gas if necessary. The uterus is located and visualized through the laparoscope. The insemination pipette is loaded with 0.3 ml of air and then the required volume of semen. Fresh semen should be stored in a water bath at 30°C until it is used. Frozen semen should be thawed just prior to use. The insemination pipette is inserted through the cannula. An avascular area of the uterine horn is identified and a stab incision is made with the pipette at a right angle to the uterine wall. The inseminator must be certain that the tip of the pipette is in the lumen of the uterus. The plunger of the insemination gun is depressed and one half of the semen is expelled. The procedure is repeated on the opposite uterine horn, and the balance of the semen is deposited. The process should be observed to determine that semen flows from the insemination gun freely without resistance and without enlargement of the tissue surrounding the stab incision. If there is any resistance to flow or if there is any indication that semen is not deposited within the uterine lumen, the position of the gun should be adjusted. After semen has been deposited in both uterine horns, the insemination gun is removed from cannula. The laparoscope and cannulae are removed and placed in the sterilizing solution in preparation for the next animal. An antibiotic spray can be applied to the wounds and a long-acting antibiotic can be injected. Does are removed to a recovery area and left undisturbed for 1 to 2 hours after insemination.

CONCLUSION

The key to success in any breeding program is sound management. Because of the many factors involved, functional and current records are essential for adequate planning and sound management decisions. Unless each factor that contributes to the percentage of does cycling and the conception rate is optimized, fertility will be reduced. Few producers can afford animals of low fertility. Any doe that fails to become pregnant under proper management conditions should be culled.

Suggested Reading

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CHAPTER 69

Clinical Reproductive Physiology and Endocrinology of Does

MARY C. SMITH

PUBERTY

Well-fed females of the European breeds commonly reach sexual maturity and begin to show signs of estrus at 6 to 8 months of age, during the autumn of their first year. If Angora doelings are small, the onset of puberty may be delayed until the second autumn. In pygmy goats, puberty may occur as early as 3 months, and occasionally even doelings of the larger breeds cycle at this young age. Introduction of a buck synchronizes puberty in a group of doelings during the breeding season. Generally, breeding should be delayed until the animal has attained 60% or more of its adult weight. Angora goats should weigh a minimum of 27 kg; larger dairy goat breeds should weigh 32 to 41 kg before being bred. If these guidelines are followed, higher conception rates and kidding percentages as well as safer parturitions will be achieved.

ESTROUS CYCLE

During the normal breeding season, August to March and especially October to December in temperate northern latitudes, goats are polyestrous. Near the equator, native breeds cycle year round but cyclicity is influenced by feed availability. Photoperiod (via secretion of melatonin from the pineal gland during hours of darkness) is an important signal for the onset of cyclicity in autumn. The end of the breeding season (transition to the anestrous period) is believed to occur because goats have become refractory to short days rather than because of a small increase in day length after the winter solstice.[1] A 60-day exposure to long days (16 hours) will end the goat's refractoriness to short days. Introduction of a buck can advance the breeding season, as most seasonally anestrous does come into estrus and ovulate within 8 days.

The normal estrous cycle of dairy goats is approximately 20 to 21 days, while pygmy goats are variously reported as having average cycles of 18 to 24 days.[2] Estrous cycles are usually more erratic at the beginning and the end of the breeding season. Short cycles of less than 12 days, and often of only 5 to 7 days, are quite common, especially in young does. Short cycles are often associated with premature regression of the corpus luteum. Estrus is occasionally observed during pregnancy.

Proestrus often lasts about 1 day. It is a period when the buck or teaser closely follows the doe, but she will not stand to be mounted. Estrus, or standing heat, lasts a variable time, often 12 to 24 hours. The doe is restless, bleats frequently, and wags her tail. Metestrus is the time from refusal to mate until formation of one or more corpora lutea. Ovulation is spontaneous and is variously reported to occur 12 to 36 hours after the onset of standing heat and may be hastened by the presence of a male goat. Diestrus, the period of corpus luteum function, is the longest portion of the cycle.

Studies in the cycling Boer doe have shown that follicular waves appear, usually with 4 waves per cycle. Follicles exceeding 3 mm in diameter emerge on days 2, 7, 12, and 17 of the 21-day cycle. There are no statistical differences between waves in number of follicles or diameter of the largest follicles.[3] Improved nutrition increases ovulation rate.

Endocrinology of the Estrous Cycle

The frequency and amplitude of luteinizing hormone (LH) pulses increase progressively as the first estrous cycle of the season approaches. Exogenous melatonin also increases LH pulse frequency. With the onset of puberty or the arrival of the breeding season, high-frequency LH pulses stimulate follicle development, there is a sustained increase in estradiol, and a preovulatory LH surge occurs, followed by ovulation. During estrus, as during seasonal anestrus, the plasma progesterone concentration is less than 1 ng/ml. Progesterone values reported during the luteal phase (typically 4 to 8 ng/ml) are variable and depend on the number of corpora lutea present and the assay procedure used. The progesterone concentration is also reduced under conditions of higher nutrition because of higher clearance rates of the hormone by the liver. The progesterone concentration drops off precipitously 3 days before the next estrus. During the last 2 days of the cycle, 17β-estradiol rises from a baseline of about 8 to 10 pg/ml to a maximum of about 32 pg/ml at the beginning of standing estrus, only to fall to baseline again 12 hours later. Peak plasma levels of LH, follicle-

stimulating hormone (FSH), and prolactin are observed during estrus, within a few hours after the estradiol peak. A second FSH peak has been detected 48 hours after the first.[4] This sequence of hormonal events is quite similar to that reported for the ewe and the cow. Inhibin is a glycoprotein hormone produced by granulosa cells that inhibits the release of FSH from the anterior pituitary. Immunization against inhibin increases the ovulation rate in goats.[5]

Estrus Detection

A teaser or breeding buck is best able to elicit and detect signs of estrus in the doe. If a buck is introduced into the herd at the beginning of the breeding season, the does will show heat in an average of 5 to 8 days. Standing and riding behavior among does is not as common as with cows. Many does will not cycle visibly unless a buck or another source of the buck odor, such as a cloth that has been rubbed all over the buck, is present in the environs. A common method of heat detection for small herds is to rub a rag on the rank buck's scent glands, caudomedial to his horns, and store this rag in a tightly covered container. The buck jar is opened and presented warm to the doe each day; when in estrus, she will be very interested in the jar. If the buck himself is present, the two animals will stay close together. If separated, they will restlessly search the perimeter of their enclosures for a means of escape.

The external genitalia may be more swollen, reddened, and more moist during estrus, but these signs are not dependable with all does. Rapid side-to-side or up-and-down tail flagging is a good sign of heat that can often be detected in the absence of a buck. The behavior probably serves to spread pheromones from the doe's vulva to any nearby males. Restlessness and a tendency to be more vocal than usual are also commonly observed. Urination may increase in frequency. Milk production and appetite may decrease and somatic cell counts in the milk increase.

Vaginal smears have been used to identify the stage of the estrous cycle with only partial success. The period of standing heat corresponds fairly well with the appearance of greater than 50% desquamated, eosinophilic, polyhedral epithelial cells in the vaginal smear. These cells decline rapidly and are replaced by more basophilic and spherical epithelial cells, which continue to be present until just before the next estrous period. Numerous metestrous leukocytes with compact nuclei appear in the smear at the time of ovulation.

A speculum examination of the cervix may be more helpful in detecting estrus. At the beginning of heat, the vaginal mucosa is reddened and moist, but little mucus is present. As heat progresses, a variable amount of transparent mucus is visible in the cervix and on the floor of the vagina. This mucus later turns cloudy and finally is cheesy white at the end of heat. Conception is best when the doe is bred at the stage at which her cervical mucus is cloudy and the cervical os is relaxed. Metestrous hemorrhage, as seen in the cow, is rarely observed in goats.

GESTATION

The placenta of the goat is cotyledonary and syndesmochorial. The caruncles are concave. The fetal trophectoderm consists of binucleate and uninucleate cells. The fetal binucleate cells migrate to and fuse with the uterine epithelium to form syncytial plaques.[6] This migration continues throughout pregnancy.

The percentage of multiple births seems to vary with the population under study, including its nutritional status. Twins or triplets are usually more common than single kids, except in primiparous animals. Quadruplets are not rare. In Angora goats, at least, the ovulation rate is 20% higher on the second heat than on the first heat of the breeding season. Fertilized ova commonly migrate to the opposite horn in multiple births, allowing better spacing of conceptuses. Transuterine migration of embryos is also common in singleton pregnancies. Implantation occurs at approximately 18 days after breeding.

Although slight breed differences do exist, the average duration of gestation is generally reported as 5 months, or 150 days (varying from 147 to 155 days). There is little effect of litter size on gestation length; quadruplets are born only 3 days earlier than single kids. Births are much more apt to occur during daylight hours than at night and are most frequent around midday.[7] If parturition occurs during the breeding season, the doe will resume ovarian activity and can conceive again.

Endocrinology of Gestation

The plasma level of progesterone, which is produced almost entirely by the corpora lutea of the pregnant goat and not by the placenta, remains high until about 4 days before parturition. Ovariectomy at any stage of gestation will cause abortion. Prostaglandin E_2 is produced in large amounts by the caprine placenta and is believed to be luteotropic in this species.[8] Several pregnancy-associated glycoproteins (PAGs) including pregnancy-specific protein B are produced by trophoblastic binucleate cells and are detectable in the peripheral circulation of the dam from 21 days after insemination and continuing throughout gestation.[9,10] Goats with two or more conceptuses tend to have higher concentrations of PAGs than goats carrying single fetuses. PAG concentrations may drop several days or weeks before abortion in conditions where placental function is disturbed.[11]

Estrone sulfate (from the fetus or placenta) begins to rise at 40 to 50 days of gestation, and can be used for pregnancy diagnosis after this time. Peak estrogen levels are reached at parturition; a very low level is found 1 day after parturition. Placental lactogen, which is also produced by the placenta and thus can be used for pregnancy diagnosis and prediction of litter size, follows a similar pattern.[12] Placental lactogen is first detectable at about 60 days of gestation and stimulates mammary gland development. Goats with false pregnancies/hydrometra will have a prolonged elevation in progesterone but will not show increases in PAGs, placental lactogen, or estrone sulfate.

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CHAPTER 70

Clinical Examination of the Female Reproductive Tract

LIONEL J. DAWSON

Goats are usually seasonal breeders. With respect to reproduction, the single most important regulating factor is the day length, or photoperiod. This influence is more pronounced the further north or south of the Equator the animal is located.

The length of the breeding season is most influenced by the following:

- Photoperiod
- Breed
- Nutrition

HOW PHOTOPERIOD INFLUENCES THE REPRODUCTIVE PROCESS IN DOES

The underlying influence of photoperiod affecting cyclicity is mediated through the secretion of luteinizing hormone (LH) from the anterior pituitary. In making the transition from anestrus to cyclicity, serum concentration of LH begins to increase.[1–9] The reason for this increase has been attributed to an increase in the frequency and magnitude of the LH pulses.[8] Final maturation and ovulation of ovarian follicles depend on sufficient LH release.[8–10]

A currently accepted hypothesis referred to as the "gonadostat hypothesis," maintains that in prepubertal and seasonally anestrus does, estrogen secretion from the follicles strongly inhibit the release of LH.[9,10] As the animal matures or is exposed to decrease in day length (late summer and fall), this inhibitory effect is lost, and there is increased LH release leading to ovulation and cyclicity.[8–10]

This change in sensitivity or alternating inhibitory influence of estrogen remains unclear. It may be due to changes in melatonin production from the pineal gland, mediated through the light perceived by the eye.[9–15]

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European goat breeds (Saanen, Toggenburg, and French Alpine), Spanish, Angoras, and Kikos have a more

restricted breeding season (September to February). Whereas Nubian, Nubian crosses, Boer, pygmy, and Tennessee stiff-legged goats have a longer or extended breeding season.[16] There is not only great variation among breeds regarding the length of the breeding season, but there are significant variations within a breed. Selection for long breeding season should be utilized as a management tool.

Nutrition and body size play an important role when doelings reach puberty. In general, doelings born in late winter or early spring will reach puberty that fall. Breeding should be delayed till animals reach 65% of their mature weight, approximately 70 lb in meat goats and 70 to 90 lb in dairy goats.[16]

Reproductive problems in goats are usually management problems associated with confinement, attempts to breed them outside their natural breeding season, improper heat detection, poor breeding management, or improper artificial insemination.[17]

CLINICAL EXAMINATION OF THE DOE

History

A complete history should be an important part of the breeding soundness examination (BSE) because of the inaccessibility to the majority of the reproductive tract to palpation or visual inspection.[16,17] Information regarding the intention of the owner in utilization of these goats for production (meat, milk, and fiber), brush control, or as a companion animal should be gathered.

Housing

Goats may be housed in a pasture, dry lot, backyard, or barn.

Nutrition

- Pasture (Bermuda, native grass)
- Supplements (concentrate, hay, silage)
- Trace minerals

Breeding Management

- Natural or artificial insemination (AI)
- Time of the year (season)
- Hormonal manipulation of the does
- Bred on natural heat or time breeding

Natural Breeding

- Buck-to-doe ratio
- Number of bucks in a pen or pasture
- Fertility of the buck used (BSE done on the bucks before the breeding season)
- Bucks' libido
- Prior history of urethral obstruction in the buck

Artificial Insemination

- Source of the semen
- Evaluation of the frozen semen
- Heat detection
- Experience and success of the inseminator
- Bred once or twice during estrus
- Evaluation of the vagina and cervix on speculum examination at the time of AI
- Presence or absence of mucus at the time of AI

Estrous Cycle (Breeding Season)

- Length of estrous cycle
- Interestrous interval
- Duration of estrus
- Reaction of the female to the male

Detection of Estrus

- Epididymectomized or vasectomized bucks with marking harnesses
- Number of does to a teaser buck
- Frequency of changing the marking crayon on the harnesses
- Number of teaser bucks checking estrous behavior among does.
- Frequency of heat detection (markings on the doe)

Other Reproductive History of the Doe

- Prior kidding history (dystocia, uterine infection, discharge, etc.)
- Average age of the flock
- Age of the doe with the reproduction problem
- Prior abortions (herd or the doe)

Other Factors

- Milking history of the dairy does
  - Milk production (305 days)
  - Daily milk production
  - Whether they are milked during the breeding season
  - Mastitis
- Any chronic infection to the doe (parasitism, caseous lymphadenitis, Johne's disease, etc.)
- Diagnosis of other diseases in the herd
- Vaccination history

Physical Examination

- Current body condition
- Conformation
- Lameness
- Polled or horned
- Eyes

External and Internal Genitalia Examination

Visual examination of the perineal area should include evaluation of the anogenital distance.

In dairy breeds, especially in Alpines, Saanens and Toggenburgs, there is a strong association between intersex and polled conditions.[18] The polled trait (P) is autosomal dominant, and the horned trait (p) is recessive;

thus, PP and Pp are polled, and pp goats are horned.[18] Current thinking is that the same or a closely linked autosomal gene controls sexual developments, but in a recessive fashion; when homozygous, this gene causes expression on the Y chromosome.[18] Thus, Pp polled goats and pp horned goats are unaffected. PP polled male goats are always sterile; PP polled female goats are genetically female (XX), become intersexes, and have impaired fertility. They often develop buck-like head and neck, buck odors, buck behavior, shortened vagina, enlarged clitoris, and an increased genital distance.[18]

Vulva

Examine the lips of the vulva, then part the lips of the vulva and evaluate the clitoris. Any lesions on the vulva (scabs, ulcers, pustules, etc.) should be noted.

Vagina

Speculum examination is made of the walls of the vagina, vestibule, and cervix. Rule out any urine pooling in the vagina and also discharge. If discharge is present, determine the source (vagina, cervix, or through the external os of the cervix).

Cervix

Examine the external os and area around the opening (fornix).

Digital examination along with speculum/endoscopic evaluation could be done to evaluate the vagina, vestibule, and cervix and to rule out persistent hymen, lacerations, and adhesions. During the period of estrus in a doe, there is clear mucus discharge seen in the anterior vagina, later turning to cloudy (milky) toward the end. This is normal discharge and does not require treatment.[17]

Reproductive Ultrasonography

Ultrasonography has been used for evaluating the reproductive tract in small ruminants.[17,19,20] Goats are scanned transabdominally either at the right or left inguinal region or may be scanned transrectally using a probe adapter to a 7.5-MHz linear transducer.

Goats are usually scanned standing, and they tolerate it better than being tipped over in a dorsal recumbency posture. Transabdominal scanning is done on the right side, slightly cranial to the mammary gland, and about 3 to 4 inches from the midline (paramedian), because the reproductive tract is pushed toward the right side by the rumen. For transabdominal scanning, 3- to 5-MHz curvilinear or sector scanning transducers are superior to linear-array transducers; 5- to 7.5-MHz linear transducers have been used transrectally[17,19,20] (Table 70-1).

Earlier pregnancies around 20 to 25 days may be seen best with a transrectal approach, preferably in a dorsal recumbency position.[22–24]

Laparoscopy and Laparotomy

Laparoscopy or laparotomy may be done in certain infertility cases in does, especially to rule out oviductal blockage, ovarian disease (cysts and tumors), and adhesions to the reproductive tract due to dystocia or surgical embryo transfers.[17]

TABLE **70-1**

Reproductive Ultrasonography in Goats[16,17,19,21–24]

Conditions	Ultrasonographic Finding
Pregnancy, 35 days to term	
30–35 days	Multiple anechoic circular areas (fluid in the coiled uterine horns); embryo and amniotic membranes could be detected in the fluid
>40 days	c- or o-shaped placentomes seen
>45 days	Fetal skeletal hyperechoic and fluid anechoic; estimating the stage of gestation could be done by measuring crown—rump, crown—nose, or fetal biparietal diameter
Pseudopregnancy	Hypoechoic uterine fluid but no embryo or placentomes
Pyometra	Hyperechoic uterine conditions but no fetal skeleton
Fetal death or mummification	No heartbeat, fluid loss or none around the fetus, +/– placentomes
Fetal maceration	Fetal parts seen, no placentomes, and less fetal fluid in the uterus; doe may have vaginal discharge and may be septicemic

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CHAPTER 71

Manipulating the Estrous Cycle in a Doe

LIONEL J. DAWSON

Goats are generally classified as "seasonally polyestrous" or short-day breeders in a temperate region. The degree of seasonality varies among breeds and their locations (latitude). Thus, the length of the breeding season is most influenced by photoperiod, breed, and nutritional status of the doe.

Native breeds near the equator generally cycle throughout the year.[1–3] In a temperate climate, goats are usually short-day breeders and have regular estrous cycles or heat cycles between August and March. But in the Northern Hemisphere in a temperate zone, the breeding season is much shorter, usually from October to December.[1,3,4]

Photoperiod and its influence on the secretion of melatonin from the pineal gland during the hours of darkness are important signals for cyclicity in the fall. The underlying influence of photoperiod, and its effects on cyclicity, is mediated through changes in pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, and the release of luteinizing hormone (LH) from the anterior pituitary gland.[5–19]

Estrus synchronization in animals that cycle throughout the year (polyestrous) focuses on manipulating either the luteal or follicular phase of their cycle. In does (seasonally polyestrous) during the breeding season, the opportunity to control their cycle is greater during the

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Native breeds near the equator generally cycle throughout the year.[1–3] In a temperate climate, goats are usually short-day breeders and have regular estrous cycles or heat cycles between August and March. But in the Northern Hemisphere in a temperate zone, the breeding season is much shorter, usually from October to December.[1,3,4]

Photoperiod and its influence on the secretion of melatonin from the pineal gland during the hours of darkness are important signals for cyclicity in the fall. The underlying influence of photoperiod, and its effects on cyclicity, is mediated through changes in pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, and the release of luteinizing hormone (LH) from the anterior pituitary gland.[5–19]

Estrus synchronization in animals that cycle throughout the year (polyestrous) focuses on manipulating either the luteal or follicular phase of their cycle. In does (seasonally polyestrous) during the breeding season, the opportunity to control their cycle is greater during the

luteal phase, which is of longer duration and is more responsive to manipulation.[22] Strategies can be employed to extend the luteal phase by supplying exogenous progesterone or to shorten this phase by prematurely regressing existing corporal lutea (CL).[20–27] Different protocols have been established for effective synchrony of the estrous cycle, and also to provide an acceptable level of fertility, when utilizing artificial insemination or natural mating. Hormones have been used in goats to manipulate the estrous cycle, but none have been approved for use on goats in the United States.

The annual reproductive cycle of goats in a temperate region can be divided into (a) breeding season, (b) nonbreeding season or physiologic anestrous period, and (c) transitional period or season.

Under natural circumstances the breeding season begins when the onset of estrus activity within a group of does is increasing, and a majority of them are exhibiting estrus. The time of the year over 90% of the group will be cycling regularly is dependent largely on the breed, location, and nutritional status of the doe. This period usually begins late August to October, and may extend up to March.

During the nonbreeding season the majority of the does are not spontaneously cycling. The time of the year depends on the breed, location, and nutritional status of the does. Blood progesterone levels are low (less than 1 ng/ml), and there is very little ovarian activity on ultrasonography. This period is usually between April and July.

The transition period is between the nonbreeding and breeding season. Does are not spontaneously cycling but can be induced to initiate estrous cycles with buck exposure or hormonal therapy. It is usually between July and August, and it depends upon the location, breed, and nutritional status of the doe. At the end of the breeding season a transitional period is believed to occur before the nonbreeding or anestrus period (March and April), when the does' breeding activity declines.[1,20,21]

During the breeding season, estrus synchronization is best done utilizing hormones either by extending the luteal phase by supplying exogenous progesterone (Table 71-1), or by shortening the diestrus phase by prematurely regressing the existing corpus luteum (Table 71-2).[20–27] Extending the luteal phase by supplying exogenous progesterone is best done by utilizing intravaginal sponges, controlled internal drug release devices (CIDR), and feed supplements.[21,22,25,26] For better synchrony of estrus and ovulation, gonadotropins and prostaglandins are used at or near the end of the progestin treatment (Tables 71-3 and 71-4). Gonadotropins commonly used are follicle-stimulating hormone (FSH) and equine chorionic gonadotropin (eCG). Equine chorionic gonadotropin is commonly used because of its longer half-life than FSH.

Table **71-1**

Extending the Luteal Phase: Progesterone or Progestagen Products Used to Synchronize Estrus in Goats[20–24,26,27,39,54]

Product	Drug	Period	Estrus
Intravaginal Sponges			
Veramix Repromap Sincrocel	60 mg medroxyprogesterone acetate (MAP)	12–14 days	24–96 hours
Cronolone Chronogest	30–40 mg flurogesterone acetate (FGA)	12–14 days	24–96 hours
Controlled Internal Drug Release Devices (CIDR)			
CIDR-G	330 mg progesterone	12–14 days	24–96 hours
Feed Supplement			
MGA	0.125 mg melengestrol	8–14 days (twice a day)	24–96 hours
	0.25 mg melengestrol	8–14 days (once a day)	24–96 hours

Note: None of these drugs are approved for use in goats in the United States.

Table **71-2**

Shortening the Luteal Phase[20–27,39–42,53]

Product	Dosage	Treatment	Route	Estrus
Lutalyse (Dinoprost tromethamine)	10 mg	Two injections, 11 to 12 days apart	IM	24–72 hours (48–60 hours)
Estrumate (Cloprostenol)	50–150 μg	Two injections, 11 to 12 days apart	IM	24–72 hours (48–60 hours)

Note: None of these drugs are approved for use in goats in the United States.

Table **71-3**

Gonadotropin Products[23,26]

Product	Dosage	Route
PG 600 5 ml contains 400 IU eCG, 200 IU hCG	Full dose, 5 ml (off season, transition, and breeding seasons)	Intramuscular
Equinex, Stimukron, Fostim, Folligon (equine chorionic gonadotropin, eCG)	400–500 IU off season and transition period 200–300 IU, breeding season	Intramuscular

Note: None of these agents has been approved for use in goats in the United States. Equine chorionic gonadotropin (eCG) mainly has FSH-like activity. Human chorionic gonadotropin (hCG) has LH-like activity.
Data in this table have been derived from Keisler DH: Endocrine control of reproduction in the ewe and ram. Proceedings of Small Ruminant Short Course (ACT and SFT), Kansas City, Mo, August 1994, pp 2–31 and Keisler DH, Buckrell BC: Breeding strategies in ewes. In Youngquist RS (ed): *Current therapy in large animal theriogenology.* St. Louis: Elsevier, 1997, pp 603–611.

The drawbacks of using eCG are as follows: higher doses many result in large numbers of anovulated follicles, and repeated usage can cause declining fertility due to the presence of antibodies against eCG.[20,21,28,33] eCG is not commercially available in the United States, but a product containing both eCG and human gonadotropin, which is labeled for use in gilts (PG600), has been tried and used successfully in goats.

TRANSITIONAL PERIOD

The buck effect is a powerful tool to enhance induction of estrus in does. Sudden introduction of previously isolated bucks to does will stimulate a surge of LH followed by ovulation in majority of does (97%) within 48 to 72 hours.[29,30,31] The initial ovulation in these animals is accompanied by estrus in 60% of the does.[29] From 30% to 60% (75%[29]) of the does will short cycle and ovulate again in 3 to 8 days or 7 to 12 days after the introduction of the buck.[20,21,27,29–31,34] Two peaks of conception have been observed after the introduction of bucks; the first one is at 7 to 12 days and the second one is about 28 to 35 days.[2,21,29] In Creole goats, which are less seasonal breeders, three peaks of conception have been observed. The first peak occurred at 3 to 5 days, the second at 8 to11 days, and the third at 27 to 29 days after the introduction of the buck.[29] This phenomenon of short cycling in late transition in does may be due to lack of prior exposure of the reproductive tract to progesterone, and the corpora lutea that form initially are short lived due to early luteal regression (ELR).[21,27,29–34] In sheep the current thinking is that early luteal regression may be due to lack of progesterone priming and the positive effects of estradiol on the uterus. Estrogen increases the availability of oxytocin receptors, leading to the release of PGF_2 alpha from these receptors, and thus lysing the corpus luteum.[32,33,34]

Does that were supplemented with progesterone (45 mg of FGA [flurogesterone acetate]) in late transition, with the sponges removed on the day the bucks were introduced, had a higher percentage of does showing estrus and reducing the number of short cycles (ELR).[31] Buck effect and exogenous hormones are methods commonly employed to induce estrus in the transitional period (Table 71-5).

NONBREEDING SEASON

Out-of-season breeding in does could be done mainly by using hormones and manipulating the photoperiod to hasten the onset of estrus. This will enable the producer to have kids from does bred out of season, take their kid crop to market when prices are high, have year-round milk production, and also increase the number of kids born to the doe during her lifetime.

A number of methods have been tried to induce estrus in this period:

1. Administering hormones (Tables 71-4 and 71-6) progesterone, eCG, and prostaglandin
2. Altering the amount of daylight the does receive during the late winter or spring (Table 71-6)
3. Treating the does with melatonin (Table 71-6)

Photoperiod manipulation is done by altering the day length. Decreasing the day length will trigger the secretion of melatonin from the pineal gland. Melatonin production may influence the secretion of LH from the anterior pituitary gland, and hasten cyclicity.[4–18] Regulating photoperiod involves the availability of lightproof barns so that the amount of light perceived by the does can be controlled. Changes in light exposure require at least 60 days to induce change or cyclicity.[35–38] Gradual change is not necessary; the amount of change that is perceived by the eye is important. A reduction from 18 to 14 hours of light per day is as effective as a reduction from 13 to 9 hours per day. It is possible to expose does to additional light in the barn, and use the outside natural light as the perceived change. For example, does are exposed to additional lights when they are brought to the barn every evening from the beginning of December. These does are exposed to about 18 to 20 hours of light (natural and artificial), and this practice is continued to the beginning of February (60 days). Then the does are not exposed to artificial lights from the beginning of February, and are exposed only to natural daylight. This will result in the perception of marked reduction in day length in February and March and will enable a large percentage of does to cycle in 6 to 8 weeks under natural light. (late March to early April).

Exogenous melatonin can be administered to supplement the endogenous release and thereby mimic short

Table **71-4**

Progesterone in Combination with Prostaglandin and Gonadotropin

Product	Dosage	Location	Duration	eCG	Prostaglandin	Season	Estrus	Breeding	Pregnancy Rate
Chronogest	20 to 45 mg FGA	Vagina	12 days	+ [48 hours prior]	+ [48 hours prior]	Breeding and nonbreeding season	24 ± 6 hours	AI 43 hours after sponge removal	70%[43]
Repromap	60 mg MAP	Vagina	16 days	+ [Removal]	—	Breeding season	32.2 ± 0.5 hours	AI 48 and 60 hours after sponge removal	52%[44]
Chronogest	40 mg FGA	Vagina	16 days	+ [Removal]	—	Breeding season	30.9 ± 0.4 hours	AI 48 and 60 hours after sponge removal	60%[44]
CIDR-G	330 mg progesterone	Vagina	16 days	+ [Removal]	—	Breeding season	27.2 ± 0.4 hours	AI 48 and 60 hours after sponge removal	47%[44]
Chronogest	30 mg FGA	Vagina	12–14 days	—	+ [Day of removal]	Breeding season	42 ± 8 hours	AI 12 and 24 hours after the onset of estrus	67%[45]
Sincrocel	60 mg MAP	Vagina	12–14 days	—	+ [Day of removal]	Breeding season	53 ± 15 hours	AI 12 and 24 hours after the onset of estrus	75%[45]
Chronogest	45 mg FGA	Vagina	11 days	+ [48 hours prior]	+ [48 hours prior]	Breeding season	33 ± 6.6 hours	Not available	Not available[46]
Chronogest	45 mg FGA	Vagina	11 days	+ [48 hours prior]	+ [48 hours prior]	Nonbreeding season	23.4 ± 7.3 hours	AI 24 hours after the beginning of estrus	75%[46,50]

Continued

Table **71-4**

Progesterone in Combination with Prostaglandin and Gonadotropin—cont'd

Product	Dosage	Location	Duration	eCG	Prostaglandin	Season	Estrus	Breeding	Pregnancy Rate
Chronogest	45 mg FGA	Vagina	11 days	+ [48 days prior]	+ [48 hours prior]	Nonbreeding season	24-72 hours [90%] in estrus	AI 45 hours after sponge removal	65%[47] if <30 hours in estrus after sponge removal; 33% if >30 hours in estrus after sponge removal
Repromap	60 mg MAP	Vagina	17 days	+ [Removal]	—	Breeding season	<48 hours 72% in estrus <72 hours 85% in estrus	Natural 24 to 96 hours after sponge removal. AI every 24 hours after the onset of estrus	71%[48] 81%[48]
CIDR-G	330 mg progesterone	Vagina	9 days	+ [Removal]	+ [Removal]	Breeding season	24–36 hours	Natural service	95%[49]
CIDR-G	330 mg progesterone	Vagina	9 days	—	+ [Removal]	Breeding season	24–72 hours	Natural service	65%[49]
Chronogest	30 mg FGA	Vagina	13 days	—	+ [Removal]	Breeding season	32.9 ± 9.7 hours	AI 12 and 24 hours after the onset	63%[51]
Sincrocel	60 mg MAP	Vagina	13 days	—	+ [Removal]	Breeding season	48.8 ± 12.0 hours	AI after the onset	65%[51]
CIDR-G	330 mg progesterone	Vagina	13 days	—	+ [Removal]	Breeding season	40.2 ± 10.5 hours	AI after the onset	63%[51]
MAP	60 mg MAP	Vagina	18 days	+ [Removal]	—	Nonbreeding season	44.6 ± 8.2 hours	Natural 24–120 hours	66%[52]

Note: Prostaglandins are not usually used during the nonbreeding season or anestrus period, because the does are not cycling.
AI, artificial insemination; CIDR, controlled internal drug release device; eCG, equine chorionic gonadotropin.

Table **71-5**

Different Methods Employed to Hasten Cyclicity During the Transition Period

Method	Duration	Estrous
1. Buck effect	Late transition	24–96 hours[2,21,29–31]
2. Progesterone (12–14 days) + equine chorionic gonadotropin (eCG) on the day or 24 to 48 hours prior to removal	Early transition	24–72 hours[54]
3. Progesterone (12–14 days) + eCG on the day or 24 to 48 hours prior to removal	Late transition	24–48 hours
4. Progesterone sponges (10 days) + eCG (removal) + prostaglandin 48 hours prior to removal	Late transition	40.9 ± 3.2 hours[55]

Note: Prostaglandins are rarely used during this period, because the does are not cycling.

Table **71-6**

Different Methods Employed During the Nonbreeding Season

Method	Duration	Estrus
1. Progesterone + equine chorionic gonadotropin (eCG) + prostaglandin	12–14 days	24–96 hours
	On the day of removal or 24 to 48 hours before	44.6 ± 8.2 hours[52]
	On the day of removal or 24 to 48 hours before	25 ± 5 hours[43,46,47,50] <72 hours = 90% estrus
2. Artificial lights	Mimic long days for 60 days followed by short days for 60 days or natural light	40–70 days[35]
3. Melatonin (implant, oral, or injection)	60–100 days	30–60 days
4. Artificial lights + melatonin	Mimic long days for 60 days followed by melatonin for 60–100 days	Buck exposure 60 days into melatonin treatment; estrus 2–3 days[36]

days associated with the onset of the breeding season.[35–38] Melatonin can be administered daily in feed, drench, injection, or a slow-release implant.[20] Melatonin is more effective in advancing the breeding season than in inducing estrus during the anestrous period.[35–38] It is more effective if its application has been preceded by long days. Thus, a combination of extra lighting for 60 days, followed by 60 to 90 days of melatonin administration, has been effective in inducing estrus.[36–38]

In a study, does receiving extra light followed by melatonin treatment for 90 days had more consistent ovulation when exposed to bucks.[36] Suggesting buck exposure was an essential component of the successful treatment (Table 71-6).

CONCLUSION

Progress has been made during the last decade toward manipulating and controlling the estrus cycle during the breeding season, nonbreeding season, and also during the transitional period. By improving the reproductive efficiency during their annual or yearly reproductive cycle, we can increase the number of kids per doe during her lifetime, producing kids out of season; kids could be sold when the prices are high; and year-round milk production in dairy animals is possible. In the United States there is limited availability of pharmaceutical products to manipulate or control the estrus cycle in does. Prostaglandins and other hormones are used in some animals to manipulate their annual cycle, but none are approved to be used in goats, although they are used in an extralabel manner (ELUD). The practitioner should be aware that under the AMDUCA regulations, nontherapeutic extralabel use is not permitted. Thus, regulatory guidance should be requested for use of these products in goats.

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CHAPTER 72

Pregnancy Diagnosis in Goats

DAVID MATSAS

Caprine pregnancy can be diagnosed over a wide range of times during gestation. Demand for pregnancy diagnosis, however, varies with herd type. Owners may simply want to know if their goats are pregnant. Commercial milk producers may justify examination costs with costs of keeping open does and the need to maintain production. Intensively managed herds benefit from early diagnosis by maximizing the number of pregnancies during a defined period. Goats bred out of season have variable pregnancy rates and diagnosis allows planning for winter milk supplies. Pregnancy diagnosis also helps monitor the success of artificial insemination.

PITFALLS TO CLINICAL DIAGNOSIS

An accurate caprine pregnancy test must distinguish hydrometra from pregnancy. Hydrometra occurs commonly in goats and may cause false positive results. Annual herd incidence varied between 3% and 21% in one study, with considerable variation among herds and within herds during different years.[1] The prevalence of hydrometra is perhaps the most important reason for pregnancy diagnosis in commercial dairy herds. The test must also be highly sensitive (few false negatives). Pregnant goats given prostaglandin usually abort. A false negative test in a doe given prostaglandin will become readily apparent.

Estrous Behavior and Physical Appearance

Goat owners frequently use clinical signs such as estrous behavior following breeding and abdominal contour to make a presumptive diagnosis of pregnancy. Failure to return to estrus after breeding during the ovulatory season suggests pregnancy, but does with hydrometra or pathologic ovarian conditions may also fail to exhibit estrus. Failure to return to estrus cannot be relied upon when does are bred late in the breeding season or during the anovulatory season. Pregnant does may show standing estrous behavior, further making this an unreliable sign. Goats with hydrometra may have large, pendulous abdomens and look pregnant. Goats with permanent stretching of the abdominal muscles (dropped stomach) have distended, pendulous abdomens and could be mistaken for pregnant.[2]

Abdominal Palpation and Ballottement

During the last half of pregnancy, the gravid uterus or fetus can sometimes be palpated through the abdominal

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Abdominal Palpation and Ballottement

During the last half of pregnancy, the gravid uterus or fetus can sometimes be palpated through the abdominal

wall. This is difficult in does that tense their abdominal muscles. Ballottement of the fetus low in the right flank, as performed in cattle, may be attempted in late pregnancy, but one is often left wondering if the "bump" felt was the ventral sac of the rumen or a fetus. Rectal-abdominal palpation with a rod has been done in goats but was too traumatic to be safely recommended.[3]

A bimanual palpation technique was recently described wherein the reproductive tract is examined per rectum by the index finger of one hand while the fingertips of the other hand are pushed upward into the ventral floor of the posterior abdomen.[4] Pregnancy was diagnosed from day 28 onward with this method. At an early stage, hydrometra cannot be distinguished from pregnancy with this technique.

Recent technologic advances have replaced many of the previously described caprine pregnancy tests.[5] Real-time ultrasonography and biologic tests are now the most sensitive and specific methods. Tests specific for pregnancy in the goat and their application relative to the day of breeding are summarized in Table 72-1.

ULTRASONOGRAPHY

A-Mode Ultrasonography

Ultrasound waves emitted by amplitude-depth units are reflected from tissue interfaces and converted to audible or visual signals. These units detect fluid-filled organs at a depth of 10 to 20 cm. Pregnancy diagnosis is based on finding fluid within the uterus, so hydrometra or pyometra will give false positive results. False negatives are possible during late pregnancy when the volume of uterine fluid relative to fetal size has diminished. Accu-

Table **72-1**

Comparison of Tests Specific for Pregnancy in Goats

Days Since Breeding	B-Mode Ultrasonography	Doppler Ultrasonography	Estrone Sulfate Test	PSPB Test	Radiography
20–25	Transrectal scan, embryo visible after day 24	NA	NA	After day 24, positive = pregnant or recent fetal death, negative = open or fetal death; high sensitivity and specificity	NA
26–35	Transabdominal scan may reveal uterus/fetus, if negative, do transrectal	Intrarectal scan may give false negative	NA		NA
36–50	Transabdominal scan: fetus, placentomes visible	Intrarectal scan	NA		NA
51–75	Same as above, may estimate age from biparietal diameter	External scan may give false negatives	After day 50, positive = live fetus, negative = open or fetal death; high sensitivity and specificity		May see fetal skeleton day 65+ but false negative possible
76–90	Same as above	External scan			Fetal skeleton visible but false negative possible
91–term	Same as above, biparietal diameter not accurate after 100 days	External scan			Fetal skeleton visible, false negative unlikely

NA, not applicable; PSPB, pregnancy-specific protein B.

racy is affected by the prevalence of hydrometra in the herd. Lack of specificity for pregnancy makes A-mode ultrasonography unreliable for pregnancy diagnosis in goats.

Doppler Ultrasonography

Ultrasound waves emitted by Doppler units are reflected from stationary and moving organs at different frequencies and converted to audible signals. Pregnancy is diagnosed by detection of the fetal heartbeat, fetal movement, umbilical blood flow, or blood flow in the maternal middle uterine arteries. Increased flow in the uterine arteries may accompany hydrometra, so pregnancy should be diagnosed on the basis of fetal sounds.

Transabdominal scans are done by placing the transducer low on the right flank. Transrectal scans are done by inserting a specially designed rectal probe into the rectum and slowly rotating it in the area of the pelvic inlet and caudal abdomen. Fetal sounds include pounding of the fetal heart, sharp fetal movements, and the swishing sound of blood flow in the umbilical arteries.[6,7] Maternal sounds include swishing blood flow in the middle uterine artery and rumbling intestinal movement.[6,7]

During the first half of gestation, transrectal Doppler was more accurate than the external approach.[6] Transrectal scans can be attempted at 25 to 30 days after breeding, but false negative results are a problem.[7] It is preferable to wait until day 35 to 40. Accuracy of transrectal Doppler was 94% to 100% for detecting pregnancy and 25% to 75% for detecting nonpregnancy in goats 55 or more days after breeding.[3] During the last half of gestation, external Doppler was nearly 100% accurate for detecting pregnancy.[6] A 2.25-MHz transducer is suggested for near-term pregnancies and a 5-MHz transducer for earlier pregnancies.[6] B-mode ultrasonography has largely replaced Doppler for pregnancy diagnosis.

B-Mode Ultrasonography

B-mode ultrasound units produce a real-time, two-dimensional image on a screen, allowing direct visualization of the uterus, fetal fluids, fetus, fetal heartbeat, and placentomes. A 5-MHz linear-array transducer is the most versatile for reproductive work in livestock. It can be used externally or intrarectally over a wide range of gestational ages. Lower frequency linear-array, sector, or curvilinear probes are also suitable for external use in goats. B-mode scanning for pregnancy can be rapidly learned, and experienced examiners achieve an accuracy of 91% to 100%.[8,9]

A transabdominal approach is generally used for examinations made more than 35 days after breeding. The standing doe is restrained against a wall or on an elevated stand or milking stanchion. The transducer is placed low on the right flank in the inguinal region. A higher quality image is obtained by clipping a small area of hair so that the probe makes direct contact with the skin. In late pregnancy, more cranial portions of the ventral abdomen may need to be shaved. An ultrasonic coupling agent is applied to the transducer head or skin to eliminate air spaces. The examiner systematically searches the caudal abdomen by slowly moving and rotating the probe in the inguinal region.

Transrectal scanning is done by inserting the lubricated probe into the rectum and slowly rotating it from side to side. Transrectal scanning is usually reserved for diagnosis at less than 30 to 35 days after breeding. Some does object at first but most acquiesce in a short time. The anechoic urinary bladder serves as a good point of orientation and is recognized by its triangular neck as the probe is moved caudally. A poor quality image or no image is usually due to feces trapped under the probe, in which case it should be removed, wiped clean, and reinserted. Limited maneuverability of the probe allows effective imaging only of the pelvic canal and an area just cranial to the pelvic brim. Alternatively, a transvaginal approach using a cylindrical sectoral probe with a variable angle head can be used to scan the reproductive tract from the cranial vagina.[10]

Pregnancy can be detected transrectally in most does by about 20 days after breeding.[9] The embryo is often visible by 24 days and is seen more consistently as gestation advances.[9] The ideal time for transabdominal scanning is roughly from 40 to 75 days of gestation when the pregnant uterus lies against the right body wall. Before day 40, the probe may have to be placed higher in the inguinal region and aimed toward the pelvic brim. Diagnosis of nonpregnancy less than 30 days after breeding is probably best achieved by the transrectal approach. Transabdominal scanning during the third trimester may require moving the probe cranially along the ventral abdomen to image the gravid uterus that has descended well into the abdominal cavity.

A positive diagnosis of pregnancy is made when the embryo, fetus, or placentomes are seen. A presumptive diagnosis of early pregnancy can be made when multiple fluid-filled sections of uterine lumen are seen cranial to the bladder (Fig. 72-1); however, hydrometra causes a false positive test. Hydrometra occurs commonly enough to advise cautious positive diagnoses until the embryo, fetus, or placentomes are seen.

During the first trimester, the embryo appears as an echogenic mass surrounded by anechoic fluid within the uterine lumen (Fig. 72-2). Seeing fetal movements or the fetal heartbeat assures viability. During the second trimester, the fetal head, thorax, and limbs become more discernible. As pregnancy advances, only portions of the fetus such as the thorax or skull may be imaged at a time. As the ratio of uterine fluid to fetus decreases during late pregnancy, it may be difficult to distinguish the fetus from the rest of the dam's abdomen. The fetal skull and beating heart are good points of orientation when scanning during late pregnancy.

Placentomes are seen by 35 to 40 days as echogenic densities in the uterine wall. By 45 to 50 days, they are more easily seen as C-shaped densities with the concave surface directed toward the uterine lumen. As pregnancy advances, numerous placentomes appear as distinct donut-shaped and C-shaped echodensities surrounded by anechoic fluid when imaged in the longitudinal and cross-sectional planes, respectively (Fig. 72-3). During the second and third trimesters, these large placentomes are

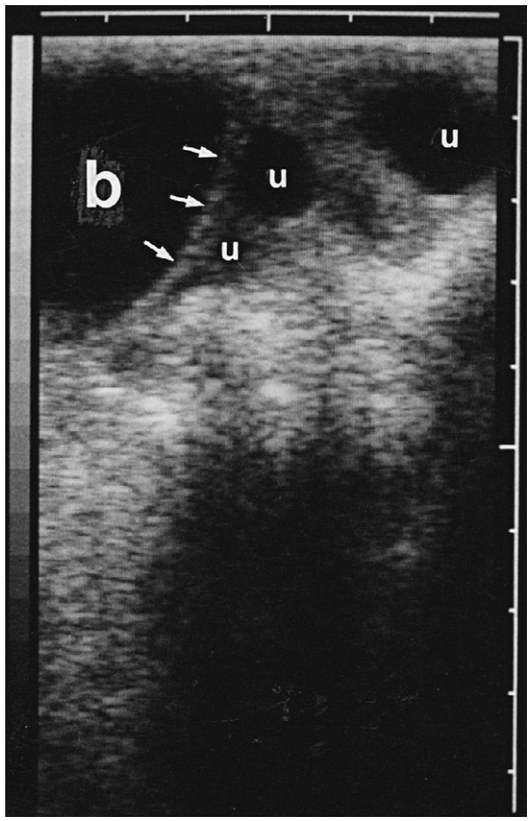

Fig. 72-1 Transrectal B-mode ultrasonographic image (produced with 5-MHz linear-array transducer) characteristic of early pregnancy (day 25). Multiple anechoic sections of uterine lumen *(u)* are present cranial to the urinary bladder *(b)* separated by the bladder wall *(arrows).* This image might also represent low-volume hydrometra, because an embryo is not seen.

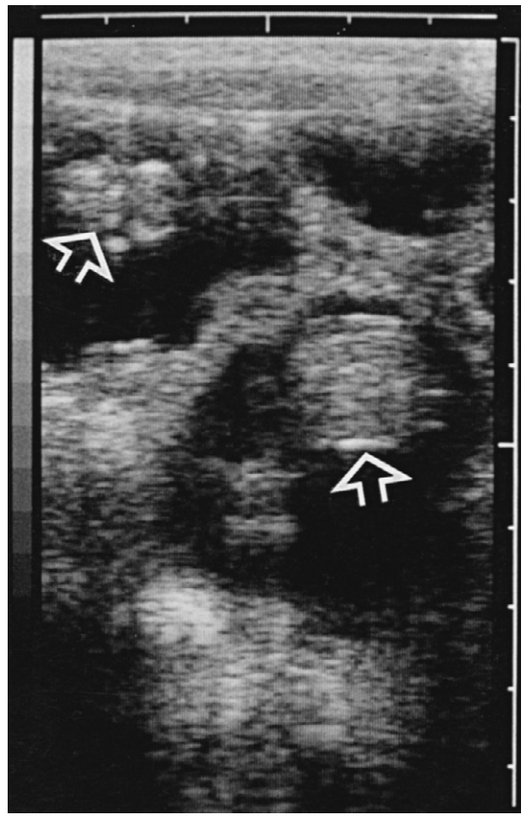

Fig. 72-2 Transabdominal B-mode ultrasonographic image (produced with 5-MHz linear-array transducer) of a 45-day twin caprine pregnancy. The embryos are seen as echogenic masses *(arrows)* within the uterine lumen. The heartbeat and movement can be seen in real time, which confirms viability.

often seen immediately after the probe is placed on the skin and allow for a rapid diagnosis of pregnancy.

Multiple fetuses can be detected to adjust feeding management. The optimal time for counting fetal numbers is between 40 and 70 days for linear-array transducers and 45 to 90 days for sector scanners.[9,11] At more than 70 days, additional fetuses may lie beyond the depth penetrated by a 5-MHz linear-array transducer. Triplets are more difficult to diagnose than twins and fetal numbers are often underestimated.

Fetal age can be estimated by measuring the width of the fetal head between 40 and 100 days of gestation.[11–14] A symmetrical image of the fetal head showing the greatest width between the parietal bones is frozen on the screen (Fig. 72-4). Symmetry is assured when both orbits are in the same image.[11] The distance between the uppermost edge of the deep and superficial parietal bones is measured with electronic calipers. Table 72-2 shows equations for estimating fetal age in various breeds based on biparietal diameter.[12–14] This technique is helpful for estimating kidding dates when breeding dates are unknown, but it requires time, patience, and practice to obtain images suitable for measurement.

Rough estimates of gestational age can be made simpler if based on uterine diameter, appearance of placentomes, and fetal size.[9,11] Placentome diameter in dairy goat breeds were correlated with gestational age up to day 90.[15] Goats were restrained over a bale of hay and transrectal measurements of placentomes in the vicinity of the bladder were made. The relationship between gestational age (GA) in days and placentome diameter (PD) in millimeters reported in this study was GA = 28.74 + 1.80(PD). Estimates of fetal age using this technique were accurate within 14 days in most of the goats studied.[15] This method was not reliable after day 90. Crown-rump length can also be used to estimate gestational age up to day 40.[9,16] A study on embryonic growth in 11 nubian goats reported a mean crown-rump length of 5.3 ± 0.3 mm on the day of first detection of an embryo (around day 20).[16] Crown-rump length increased an average of 1.4 mm per day, reaching 34.2 ± 0.6 mm on day 40.

A clear advantage of B-mode ultrasonography is the ability to distinguish a viable pregnancy from hydrometra, pyometra, or fetal mummification.[11] In hydrometra, the uterus appears distended with anechoic fluid, and no fetus or placentomes are identified (Fig. 72-5).

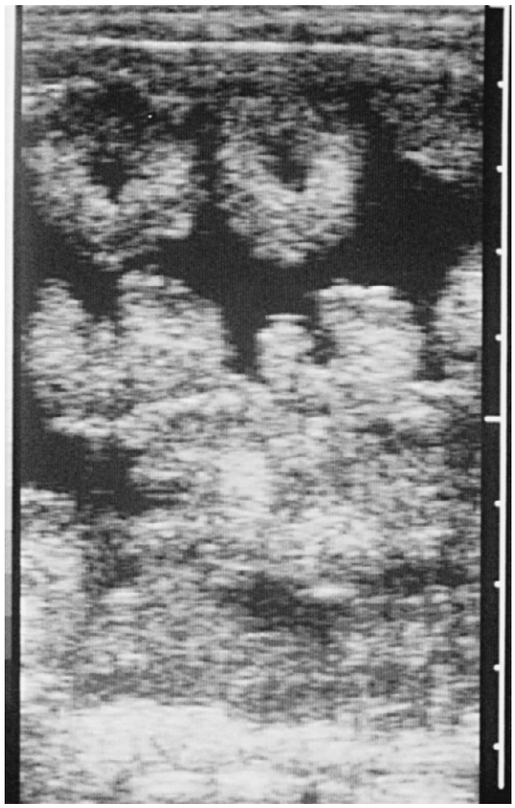

Fig. 72-3 Transabdominal B-mode ultrasonographic image (produced with 5-MHz linear-array transducer) of a 90-day caprine pregnancy. Distinct placentomes appear as donut-shaped (longitudinal plane) and C-shaped (cross-sectional plane) echodensities surrounded by anechoic fluid.

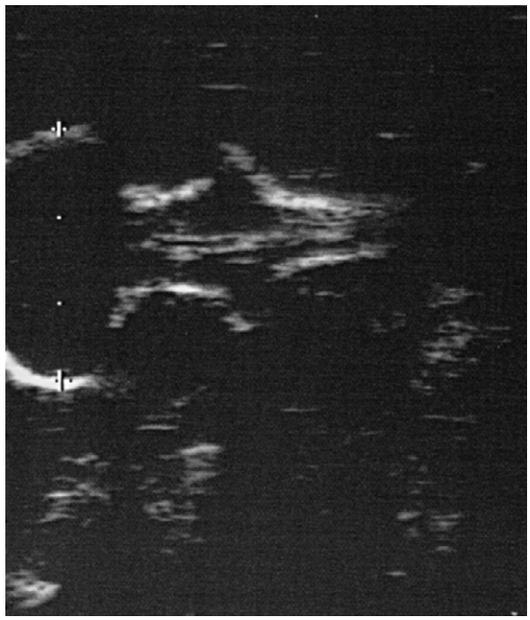

Fig. 72-4 Symmetrical transabdominal B-mode ultrasonographic image (produced with 5-MHz linear-array transducer) of a fetal head. Biparietal diameter measured with electronic calipers was 29 mm. Gestational age in this Nubian doe was estimated to be 77 days using the equation shown in Table 73-2.

Table **72-2**

Relationship Between Gestational Age and Fetal Biparietal Diameter*

Breed	Gestational Age (Days)
Toggenburg	27.9 + (1.64 × BPD)
Nubian	26.8 + (1.74 × BPD)
Angora	28.6 + (1.77 × BPD)
Pygmy	23.2 + (2.08 × BPD)

*Biparietal diameter (BPD) is measured (in mm) transabdominally using real-time ultrasonography with a 5-MHz linear-array transducer.
Data from Haibel GK, Perkins NR, Lidl GM: Breed differences in biparietal diameters of second trimester Toggenburg, Nubian, and Angora goat fetuses. *Theriogenology* 1989;32:287; and Reichle JK, Haibel GK: Ultrasonic biparietal diameter of second trimester pygmy goat fetuses. *Theriogenology* 1991;35:689.

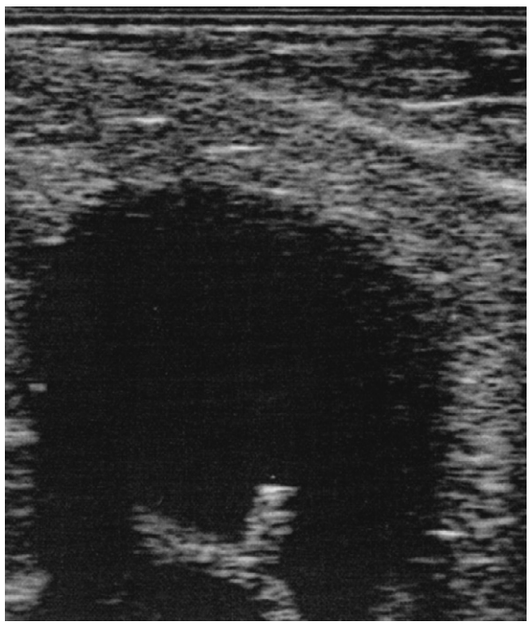

Fig. 72-5 B-mode ultrasonographic image (produced with 5-MHz linear-array transducer) of a hydrometra in a doe. The anechoic fluid-filled uterus is void of placentomes. The Y-shaped echodense lines at the bottom of the image represent the folded uterine wall.

Intersecting echodense lines representing the folded uterine wall are usually seen in the image. In pyometra, the uterus appears distended with a gray to white echoic fluid, and no fetus or placentomes are visualized. A mummified fetus appears as a dense, hyperechoic image lacking surrounding fluid with no heartbeat or placentomes.

BIOLOGIC TESTS

Estrone Sulfate Test

Estrone sulfate is a pregnancy-specific hormone produced by the fetoplacental unit in increasing amounts as pregnancy advances. After day 50 after breeding, there is a significant difference in serum and milk estrone sulfate concentrations between pregnant and nonpregnant does.[17–19] Accuracy for detection of pregnancy and nonpregnancy is high any time after 50 days after breeding. A positive test suggests that the fetus was alive at the time of sampling. A positive correlation between estrone sulfate concentration and fetal numbers was found between 51 and 65 days and after 90 days of gestation, but the concentrations were too variable to be of practical value.[19] Hydrometra will not cause false positive results with this test. False positives may occur with hemolyzed serum samples.[17] False negative tests may occur if samples are collected before 50 days of gestation. False negatives are theoretically possible in correctly timed samples, so cautious prostaglandin use is advised following a negative test.

Estrone sulfate can be measured in blood, milk, or urine by radioimmunoassay.[7,17–19] A urine test that measures total estrogens is available as a goat pregnancy test.*

Pregnancy-Specific Proteins

Pregnancy-specific protein B (PSPB) and pregnancy-associated glycoproteins (PAG) have been identified in various ruminant species including cattle, sheep, and goats.[20,21] They are produced by the placenta throughout gestation and can be measured in plasma by radioimmunoassay. Plasma PSPB and PAG concentrations were significantly different between pregnant and nonpregnant goats by day 24 and day 21, respectively.[20,21] Low levels of PSPB (<1.1 ng/ml) were found in nonpregnant goats, possibly due to cross-reactivity of antiserum with serum proteins.[20]

PSPB and PAG tests are highly specific for pregnancy. Blood levels of these proteins may remain elevated for a short time after embryonic or fetal death, which could explain apparent false positive results. Hydrometra will not cause false positives. As with all biologic tests, false negative results are possible, so cautious prostaglandin use following a negative test is warranted. A laboratory serum PSPB pregnancy test is available that is greater than 95% accurate when goats are tested after day 25 after breeding.† Future possible development of either of these tests into an on-farm test kit would offer a practical alternative to ultrasound diagnosis.

Progesterone Test

Progesterone tests more accurately detect nonpregnancy than pregnancy. Goats depend on progesterone from the corpus luteum to maintain pregnancy throughout gestation. Plasma progesterone concentrations above 1 ng/ml suggest functional luteal tissue and remain elevated throughout pregnancy. Progesterone is not pregnancy-specific, however, and elevated concentrations may accompany hydrometra, pyometra, early embryonic death, and fetal mummification, causing false positive results. Plasma progesterone concentrations below 1 ng/ml suggest nonpregnancy.

The best use of progesterone tests is to identify nonpregnant does 3 weeks after breeding. Does with low progesterone concentrations are considered open and are scheduled for rebreeding. This is particularly useful for does bred out of season, which do not reliably exhibit estrus after an infertile service. Accuracy of diagnosis from blood or milk progesterone levels, 20 to 24 days after breeding, varied from 80% to 100% for nonpregnancy and 67% to 100% for pregnancy.[22–25] Concentrations of progesterone in blood used for classifying nonpregnancy varied from less than 1.5 to less than 2.5 ng/ml.[22,23] Concentrations of progesterone in milk varied considerably among pregnant goats and among herds.[22] Nonpregnant does returning to estrus may have high milk progesterone concentrations for 1 to 2 days following lysis of the corpus luteum.[26] Plasma progesterone concentrations more accurately reflect the true endocrine status of does and were more accurate than milk progesterone concentrations for pregnancy diagnosis.[22] Does with blood progesterone concentrations between 1 and 2 ng/ml are difficult to classify accurately.

Progesterone testing has been simplified by on-farm enzyme immunoassay kits, although none are currently marketed for goats. Use of these kits with caprine blood and milk has generally given accurate results.[24,25] The accuracy of kits marketed for other species should be validated for goats before use.

In summary, progesterone testing for pregnancy diagnosis is plagued by the problem of false positive results. High progesterone concentrations only indicate the presence of functional luteal tissue. Low concentrations any time after days 4 to 6 after breeding suggest nonpregnancy.

RADIOGRAPHY

Abdominal radiography is useful for detection of pregnancy and determination of fetal numbers from the latter part of the second trimester until term. Diagnosis is based on detecting the fetal skeleton. The fetal skeleton may be seen by 58 days after breeding but is more consistently radiopaque after the 65th day.[27] Uterine enlargement suggestive of pregnancy may be seen earlier but cannot be differentiated from hydrometra or pyometra. It is recommended to wait 90 days after breeding to make a radiographic diagnosis of nonpregnancy.[7] The limits of small

*BET Reproductive Laboratory, Lexington, KY.
†BioTracking, Moscow, ID.

animal radiographic equipment may be exceeded or nearly exceeded by large does. Waiting until 90 days will make false negatives due to suboptimal technique less likely and avoid the need for repeated examinations. Hydrometra can also be diagnosed at this time if no fetal skeleton is seen.

ERRORS IN DIAGNOSIS

Understanding the common sources of error for each test will reduce the number of false positive and false negative results. False negatives (negative result in a pregnant doe) can have the most serious consequences if prostaglandin is administered. False positives (positive result in a nonpregnant doe), besides causing owner disappointment, delay the time to rebreeding or culling and so are economically unsound. With all tests, apparent false positives (true positive result but doe never kids) could be explained by unobserved abortions or embryonic or fetal death after the test was done.

Ultrasonographic Errors

A-Mode

The inherent problem with A-mode is the inability to distinguish pregnancy from hydrometra, pyometra, or the urinary bladder. False negatives are possible during late pregnancy when the volume ratio of uterine fluid to fetus is diminished. Other tests are recommended for accurate pregnancy diagnosis in goats.

Doppler

Accuracy for classifying nonpregnancy (specificity) is difficult to define because of the small number of open does included in previous studies.[3,6] Positive pregnancy diagnoses based only on fetal sounds (heartbeat or umbilical artery blood flow) should eliminate false positive errors. Correct interpretation of fetal sounds could be a problem for operators inexperienced with Doppler units. Fetal pulses in the umbilical arteries are faster than maternal pulses. Increased blood flow in the maternal uterine arteries does not necessarily indicate pregnancy.

After day 75, transabdominal Doppler is a highly sensitive pregnancy test. False negatives may occur under day 75 with the transabdominal approach. The transrectal approach is probably more accurate under day 75. Under day 40, false negatives may be a problem.[7] False negatives may also occur when feces around the rectal probe interfere with wave transmission. This should be suspected when no sounds at all are heard.[7]

B-Mode

False positive errors can be eliminated by making a positive pregnancy diagnosis only after seeing placentomes or the embryo or fetus surrounded by anechoic uterine fluid. Adherence to this rule should preclude misdiagnosis of hydrometra as pregnancy, especially during early examinations. The urinary bladder should not be misinterpreted as a fluid-filled uterus. The bladder is differentiated by its triangular neck. Intestinal loops imaged in cross section may appear as rows of C-shaped echodensities and should not be misinterpreted as placentomes. Placentomes produce a discrete echoic image surrounded by anechoic fluid.

False negatives result from failure to image an early pregnancy, an incomplete scan of the abdomen, failure to distinguish a near-term fetus from the rest of the dam's abdomen, or operator inexperience. In a transabdominal scan of a doe bred less than 40 days, the area just cranial to the pelvis must be thoroughly imaged. A transrectal scan during this time should lessen the likelihood of missing an early pregnancy.

Biologic Test Errors

Estrone Sulfate and Pregnancy-Specific Proteins

These tests are highly sensitive and specific for pregnancy in the goat, but it must be recognized that no biologic test is 100% accurate. In this regard, there is nothing like visualizing a pregnancy in real-time with B-mode ultrasonography. Errors may arise from the test methodology itself, improper handling of samples, or improper timing of sample collection. Inaccurate or unknown breeding dates may cause erroneous interpretation of results. If an exact breeding date is unknown or continuous buck exposure has occurred, a negative estrone sulfate or PSPB test has little meaning. The possibility of prostaglandin-induced abortion caused by a false negative must be considered.

Progesterone

A positive pregnancy diagnosis cannot be made solely from a high progesterone concentration. Accuracy for detecting nonpregnancy depends on the absolute concentration chosen to distinguish pregnancy from nonpregnancy. Discriminating blood concentrations are generally in the 1 to 2 ng/ml range. Results between 1 and 2 ng/ml progesterone are equivocal and such goats may be pregnant or open. Milk progesterone concentrations are even more variable and may differ among herds, as previously discussed. Errors could also result from the test methodology itself.

Radiographic Errors

Observation of the fetal skeleton will eliminate false positives. Hydrometra and pyometra cause false positives if diagnosis is based on uterine enlargement alone. False negatives are due to poor technique or examination before 90 days after breeding. Poor technique is exacerbated by doing the examination too early. Unknown or inaccurate breeding dates could account for false negatives if only a single examination is performed. If the breeding date is questionable and a negative radiographic result is obtained, re-examination at a later date is indicated.

SUMMARY

B-mode, real-time ultrasonography is the best overall method of pregnancy diagnosis in goats. Externally applied Doppler ultrasonography is a highly sensitive pregnancy test after day 75 of gestation. If ultrasonography is unavailable or impractical, the best alternative is

the estrone sulfate or PSPB test. Progesterone testing may aid early detection of nonpregnancy. Abdominal radiography performed 90 or more days after breeding is an accurate test for individual does.

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CHAPTER 73

Parturition and Dystocia in the Goat

WILLIAM BRAUN, JR.

PARTURITION

Parturition is signaled by a series of events in pregnant does that alert the owner to impending kidding. Relaxation of the pelvic ligaments, as a result of an increase in circulating estrogen levels in late gestation, warns the owner that kidding is imminent. At about the same time, the vulva enlarges and may become slightly longer. In most goats there is a rapid enlargement and engorgement of the udder as well, although udder enlargement may not be an accurate indicator of impending parturition, as first fresheners may initiate udder enlargement as early as the third or fourth month of gestation. Some does have early engorgement of the udder to the point that milking is required. Other does do not show udder enlargement until the point of, or just shortly after, parturition.

As in other species, parturition is divided into three stages. The first stage is the initiation of myometrial contractions as progesterone levels drop and estrogen levels increase. In first fresheners, this stage may last from 2 to 12 hours. Pluriparous does may show very little discomfort, and the first stage may last only a few hours. The doe is restless and most signs are reflective of abdominal discomfort. She will lie down and rise, paw at the bedding, and maybe urinate or defecate in frequent, short bouts. During this period, the cervix relaxes and releases the cervical seal that is seen at the vulva as thick, tenacious, yellowish brown mucus. The contracting uterus pushes the placenta, fetus, and fetal fluids toward the cervix, further dilating it.

When the placenta and its contents fully dilate the cervix and come into contact with the vagina, second stage labor is initiated with the concomitant abdominal press that is characteristic of active labor. Depending on the number of fetuses present, this stage will last from 1 to 3 hours, with most does delivering all fetuses within 2 hours. Does typically are in lateral recumbency during this stage, but some older, more experienced does may remain standing for delivery. After the placenta reaches the vagina, the chorioallantois ruptures, lubricating the vaginal canal, and the amnion, or water bag, is partially delivered through the vulvar opening. This also ruptures and the kid is delivered. In multiple births, the doe may rest between deliveries, or they may occur one after the other.

Third stage parturition is characterized by delivery of the placenta or placentas and involution of the uterus. In the goat, the placenta is usually delivered within 1 hour of kidding and is considered retained if not expelled by 12 hours. In some multiple births, placental expulsion may be intermingled with delivery of kids. The mass and volume of the postpartum uterus decline drastically from delivery until about day 12 post partum.[1,2] Involution is macroscopically complete by day 28 post partum. The rapid decline in mass and volume of the uterus is ascribed to contraction of the myometrium together with vasoconstriction and loss of tissue fluids.[2] Lochia is normally discharged for up to 3 weeks. This is a nonodorous, reddish brown discharge that represents the debris remaining in the uterine lumen from parturition as well as some of the residual tissue and fluid from uterine involution.

DYSTOCIA

Normally, delivery is uneventful in goats. Dystocia exists when delivery is prolonged or some event occurs that makes delivery difficult or impossible. The number of cases that require obstetric assistance is low, with only 3% to 5% of births requiring help.[3] Most birthing problems are handled by owners and only the more difficult cases are submitted for veterinary assistance. Dystocia is considered to exist if the doe has been in active labor for 30 minutes or longer and is not making progress toward delivery of the kids.[4]

Most kids are born in cranial, longitudinal presentation, dorsosacral position with extremities extended, like cattle. Some 3% to 9% of kids are born in caudal, longitudinal presentation, almost all in sets of twins or multiples.[3] The incidence of dystocia is higher, on a percentage basis, for births in caudal presentation in goats.[3] In caudal presentations, 80% of the single births had one or both hindlimbs flexed.[5]

The most common dystocia arises when more than one fetus tries to exit the vaginal canal at the same time.[4] This tangle of kids is often separated by skilled owners. Other causes of dystocia are deviations from normal presentation, position, or posture, fetomaternal disproportion, failure of cervical dilation (ringwomb), vaginal prolapse, uterine torsion, and uterine inertia. Dystocias are commonly seen in Nubians because of multiple births; in first freshener Saanens that often have a single, large fetus[6]; and in pygmy goats that are too short-coupled to allow the fetus to properly position itself. One study reported that of 43 cases of fetal dystocia, 3 were caused by fetal oversize, 29 by disposition errors, and 11 by simultaneous presentations.[7]

Case Management

The diagnosis of dystocia is based on the owner's observation of the kidding process. This observation may include failure of active labor to be initiated after an appropriate time span, prolonged labor without producing kids, abrupt cessation of parturition, prolapse of portions of the reproductive tract, delivery of the placenta without delivery of kids, or a sense of anxiety that all is not going well. The veterinarian should supplement the owner's observations with historical information such as expected due date, problems during previous kiddings, parity of the doe, assistance already rendered, and any other information that the circumstances may warrant. Many owners have more experience in handling caprine dystocias than the veterinarian, and if assistance is requested, it is often in cases that require cesarean section. The length of time that the doe has been in labor is important. The cervical canal appears to remain dilated for a much shorter time period in goats than in sheep.[3] If delivery has not been accomplished within 2 to 3 hours, the cervix starts to close.

Examination of the doe and all manipulative procedures should always follow the general principles of veterinary obstetrics: cleanliness, lubrication, and gentleness. Does can be restrained in a stanchion or by a halter, or held by the owner. The perineal area is cleansed with soap and water, as are the hands and arms of the obstetrician. Examination of the reproductive tract in goats can be difficult because of the small size of the animal. Epidural anesthesia may be beneficial to prevent straining during examination or the subsequent manipulations. Some large breeds of goats will accommodate manual exploration of their tract all the way into the uterus, if the obstetrician has hands and arms of an appropriate size and plenty of lubrication is employed. Pygmy goats are more problematic and may only allow digital exploration of the vagina. In some cases in which no fetus or placenta is presented, a speculum examination of the vagina is in order. In any event, extreme care must be exercised, as the uterus and vagina are fragile and can be easily ruptured. After physical and visual examination, a diagnosis of the cause of dystocia can be made and a plan formulated for relief. The relief of dystocia includes untangling multiple kids, mutation of maldispositions, traction, partial fetotomy, and cesarean section.

Mutation and Traction

Most cases of simultaneous presentation are corrected by the goat's owner without veterinary assistance. Some novice owners will, however, present these cases for delivery. Patience in determining which extremities belong to which kid and untangling them is usually all that is required. A leg or head snare that permits a better grasp of the extremities is a useful item in these cases. Once the extremities of the first kid are identified, the second fetus is gently repelled back into the uterus and the first kid delivered by traction.

Flexion of the neck, carpus, and shoulder are the most common maldispositions in goats.[7] Not all cases of abnormal fetal presentation, position, or posture result in dystocia. Some does may kid normally if only one forelimb is presented with the head.[6] In dystocias caused by a relatively large head blocking the vaginal canal, one of the forelimbs may be repositioned into shoulder flexion, allowing room for passage of the head and remaining forelimb, and the kid delivered by traction. In any case in which the vagina contains fetal parts, more room can be created for mutation by gently repelling the fetus back into the uterus. This may also be accomplished by elevating the rear quarters of the doe and letting gravity assist in repositioning.

A kid in cranial presentation should never be delivered by traction if the neck is flexed and the head retained, as this may cause rupture of the birth canal. If sufficient room is available, the fetus can be repelled back into the uterus and the head repositioned by hand or with the aid of a head snare. In some cases, just the head is presented and both shoulders are in a flexed position. In small fetuses, traction on the head with a snare may be sufficient for delivery after the vaginal canal has been well lubricated. Dystocia after delivery of the head through the vulva may result in passive congestion of the head from pressure placed on the vessels of the neck by the vulva and vagina. Passive congestion will quickly resolve following delivery.

Carpal and shoulder flexion are corrected similarly. Carpal flexion is corrected digitally by hooking a finger around the distal forelimb below the flexed carpus and straightening the limb. Care is exercised to prevent laceration of the vaginal canal during repositioning. For shoulder flexion, the operator's hand is passed along the affected side of the head and neck until the shoulder joint is reached. A finger is hooked around the upper forelimb and the limb mutated into carpal flexion position. The carpal flexion is then corrected. It may be necessary to repel the fetus slightly into the uterus to accomplish this task. Hip and hock flexion in fetuses in caudal presentation are handled similar to shoulder and carpal flexion.

Fetotomy

A complete fetotomy, as in cows, is rarely attempted in goats. Partial fetotomy of the extremities is successful for relief of some dystocias. Fetotomy instruments for goats usually consist of a finger knife or scalpel blade and handle. Of prime concern when performing a fetotomy is that the fetus is already dead or the owner is willing to sacrifice a poorly viable fetus.

The most common reason for a partial fetotomy is amputation of the head to allow more room in the vagina for further manipulations or space for passage of the remaining fetal parts. When the head has been delivered through the vulva, it is relatively simple to amputate the head and as much of the neck as possible. In cases of head or neck flexion, it may be necessary to apply some traction to the fetus in order to get a scalpel blade in proper position to amputate the head.

Forelimbs are usually removed by subcutaneous fetotomy. A scalpel is used to circumferentially incise the skin around the carpus and then the incision is extended

medially along the leg as far as possible. The skin is bluntly dissected from the limb and the limb rotated to separate it from the body. The other forelimb is removed in a similar manner. To remove the thorax, the operator's hand is passed beneath the fetal skin to a point where the ribs can be manually crushed. The thorax is then rotated, causing the lumbar vertebrae to separate, and the cranial portion of the fetus is removed. The hindlimbs can then be removed.

Fetomaternal Disproportion

Fetomaternal disproportion may be of either fetal or maternal origin. Fetal disproportion results when a fetus is relatively too large to pass easily through the maternal pelvis or vaginal canal. This is most often encountered in does carrying a single fetus. Only parts of the fetus are capable of being delivered into the vaginal canal. If a live birth is expected, cesarean section is the preferred method of delivery. With adequate lubrication and judicious application of traction, some large fetuses can be delivered vaginally. Other less common causes of fetal oversize include anasarca or fetal hydrops and dead emphysematous fetuses. Both of these conditions can be relieved by incising the fetus to release some of the accumulated fluid or gas, by partial fetotomy, or by cesarean section.

Disproportion of maternal origin is most common in small first fresheners that have not adequately grown by the time of parturition. The pelvis is not large enough to allow easy passage of the fetus, which may be a large singleton. Other conditions that cause a relatively small pelvis or birth canal in does include injuries involving pelvic fractures, ankylosis of the tail, and soft tissue enlargements around the vagina such as abscesses or tumors. If lubrication and traction will not relieve this type of dystocia, a cesarean section will be needed for live deliveries.

Cervical Dilation Failure

Incomplete cervical dilation, or ringwomb, is a relatively common cause of caprine dystocia, accounting for 23.5% of all dystocia cases in one study.[8] Some predisposing factors include hypocalcemia, hormonal or mineral imbalances, twinning, season, and breed. Prolonged dystocia often results in the previously dilated cervix closing, further complicating the dystocia. Partial dilation of the cervix results in the cervical canal opening only a few centimeters instead of complete dilation. The myometrium pushes the fetus against the partially open cervix and then fatigues. Secondary uterine inertia is a common sequela to ringwomb.

Does are presented to the veterinarian because no kids have been delivered or only the placenta has been partially delivered. Digital examination reveals a partially dilated cervix with the cervical rings palpable. Often the canal is open only enough to allow a couple of fingers to be introduced. Attempts at manual dilation are unrewarding in most cases, with tearing of the cervix or uterus as possible sequelae. If attempts are made at manual dilation, patience and great quantities of lubricant should be employed. Treatment is typically by cesarean section. If hypocalcemia is suspected as the underlying cause, supplementation with calcium borogluconate (60–100 ml IV) may be beneficial.

Uterine Torsion

Torsion of the uterus is an occasional cause of dystocia in goats. In one report, uterine torsion accounted for 41.5% of the cesarean sections performed at a referral center.[9] Torsion typically occurs in does carrying a single fetus. Presenting signs in does with uterine torsion may mimic cervical dilation failure. Diagnosis is complicated by the inability to perform rectal palpation. Vaginal examination may reveal the typical folds in the vaginal wall or may reveal a cervix that is only partially dilated. Often diagnosis and treatment are performed at the same time with a cesarean section. If torsion can be diagnosed, rolling the goat, as in cows, may correct the torsion, but then time must be allowed for the cervix to dilate to achieve a vaginal delivery.

Uterine Inertia

Uterine inertia may be the cause or the result of dystocia. Primary uterine inertia results from failure of the uterus to properly contract during parturition. The cause is suspected to be hypocalcemia, a hormonal imbalance at parturition, or an inability of the myometrium to properly respond to contraction signals. Secondary uterine inertia is the result of failure of contractions to empty the uterus, causing fatigue of the myometrium. This may be caused by incomplete cervical dilation, maldispositions, or conditions that block the birth canal. Depending on the underlying cause, relief is by mutation and traction, supplementation with calcium, or cesarean section. Oxytocin (10–20 IU) may be given in an attempt to stimulate contractions of the uterus, but response is usually poor in cases of uterine inertia.

Aftercare

Aftercare following dystocia depends on the type of assistance rendered. If only additional traction was needed for delivery without invasion of the reproductive tract, little or no special care need be provided. In cases in which the reproductive tract has been invaded, systemic antibiotics may be warranted. Most commonly this consists of procaine penicillin G (22,000 IU/kg twice a day, IM or SQ) or oxytetracycline (11 mg/kg twice a day, IV, IM, or SQ) for 3 to 5 days post partum, with penicillin being the preferred choice. Because goats are susceptible to tetanus, a tetanus toxoid booster or tetanus antitoxin may be warranted. Oxytocin (10–20 IU IM, SQ, or IV) is administered to stimulate uterine contractions to help evacuate the uterus or to control reproductive tract hemorrhage.

In cases of fetotomy or when gross contamination of the uterus is suspected, uterine lavage with warm saline or water to which a mild antiseptic has been added may be necessary. A clean stomach tube and funnel or calf esophageal feeder can be used to lavage the uterus. Lavage

flushes debris from the uterus and stimulates uterine contractions. Antibiotic boluses can also be inserted into the uterus at this time.

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CHAPTER 74

Postpartum Care of the Doe and Kid

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PREPARTUM PREVENTION OF POSTPARTUM PROBLEMS

Prevention of postpartum problems in does and kids begins long before parturition. Does should be examined with ultrasonography to confirm pregnancy status and stage of gestation, their body condition score (1 to 5) should be determined, and lactating does should be dried off 60 days before the expected due date. Failure to observe a 60-day dry period results in modest to marked decreases in milk production during the ensuing lactation period. An inadequate dry period may also result in decreased colostrum quality.

Some does are difficult to dry off without damage to the udder when the traditional method is used of sudden cessation of milking, withholding of water for 12 to 24 hours, decreasing of forage quality, withholding of concentrates, and isolation from the environmental stimuli that does associate with the milking procedure. The producer can dry these does off more slowly by reducing them to once-daily milking for 2 to 3 weeks prior to the expected dry-off date and decreasing concentrate consumption, followed by traditional methods of ending the lactation. An additional complication in drying off does is the inability to switch them to low-quality forage early in their dry period, for they often remain in their normal social and parity group. Removal and reintroduction of does into milking strings may lead to prolonged social aggression, resulting in significant loss of milk production and occasionally injury to does. An alternative is to breed and dry off does as a string so that feed adjustments commensurate with the varying nutritional needs of the production cycle can be made. During the last 2 to 4 weeks of the dry period, does should be fed according to body condition score, with thin does (<3.0 on a 5-point scale) receiving increased concentrate for 4 weeks over the basic 0.45 to 0.90 kg offered to does in good to overconditioned state. Ideally, does should freshen with a body condition score of 3.5 to 3.75 (on a 5-point scale). Concentrate feeding during the latter part of the dry period allows for a smooth transition from pregnancy to lactation during which high-energy feeds are required to optimize production and maintain an acceptable calcium–to-phosphorus ratio of not more than 1.5:1. Does should be closely watched during the last 4 to 6 weeks of the dry period for signs of ketosis, hypocalcemia, or abortion. Vaccination of does during late gestation for enterotoxemia and tetanus will optimize the specific immunoglobulins present in colostrum.

Maintenance of a clean, draft-free kidding area is critical for optimal doe and kid health. Kids are born agam-

flushes debris from the uterus and stimulates uterine contractions. Antibiotic boluses can also be inserted into the uterus at this time.

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CHAPTER 74

Postpartum Care of the Doe and Kid

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PREPARTUM PREVENTION OF POSTPARTUM PROBLEMS

Prevention of postpartum problems in does and kids begins long before parturition. Does should be examined with ultrasonography to confirm pregnancy status and stage of gestation, their body condition score (1 to 5) should be determined, and lactating does should be dried off 60 days before the expected due date. Failure to observe a 60-day dry period results in modest to marked decreases in milk production during the ensuing lactation period. An inadequate dry period may also result in decreased colostrum quality.

Some does are difficult to dry off without damage to the udder when the traditional method is used of sudden cessation of milking, withholding of water for 12 to 24 hours, decreasing of forage quality, withholding of concentrates, and isolation from the environmental stimuli that does associate with the milking procedure. The producer can dry these does off more slowly by reducing them to once-daily milking for 2 to 3 weeks prior to the expected dry-off date and decreasing concentrate consumption, followed by traditional methods of ending the lactation. An additional complication in drying off does is the inability to switch them to low-quality forage early in their dry period, for they often remain in their normal social and parity group. Removal and reintroduction of does into milking strings may lead to prolonged social aggression, resulting in significant loss of milk production and occasionally injury to does. An alternative is to breed and dry off does as a string so that feed adjustments commensurate with the varying nutritional needs of the production cycle can be made. During the last 2 to 4 weeks of the dry period, does should be fed according to body condition score, with thin does (<3.0 on a 5-point scale) receiving increased concentrate for 4 weeks over the basic 0.45 to 0.90 kg offered to does in good to overconditioned state. Ideally, does should freshen with a body condition score of 3.5 to 3.75 (on a 5-point scale). Concentrate feeding during the latter part of the dry period allows for a smooth transition from pregnancy to lactation during which high-energy feeds are required to optimize production and maintain an acceptable calcium–to-phosphorus ratio of not more than 1.5:1. Does should be closely watched during the last 4 to 6 weeks of the dry period for signs of ketosis, hypocalcemia, or abortion. Vaccination of does during late gestation for enterotoxemia and tetanus will optimize the specific immunoglobulins present in colostrum.

Maintenance of a clean, draft-free kidding area is critical for optimal doe and kid health. Kids are born agam-

maglobulinemic, so the magnitude of initial pathogen challenge will influence neonatal morbidity and mortality. Incidence of postpartum metritis and mastitis in does is minimized if build-up of environmental organisms is avoided. Factors affecting environmental challenge (persistence and replication of pathogens in the environment) include population density, ventilation, and maintenance of clean, dry bedding areas. Overcrowding in the maternity area and in the area housing neonatal kids increases the build-up of fecal organisms. Environmental pathogens proliferate under warm, moist conditions. Soiled bedding in the maternity area should be removed and replaced frequently. Adequate ventilation and exposure to sunlight will reduce pathogen numbers. Inadequate drainage promotes moist conditions. Shelter from wind, rain, and other inclement weather reduces stress on the doe and is critical for kid survival. Kids exposed to extremely hot or cold weather conditions, particularly during the first 72 hours of life, have increased mortality rates.

PARTURIENT DOE MANAGEMENT

Does should be observed closely as they near kidding. Clipping hair from the udder, tail, and perineal area helps prevent contamination of the reproductive tract during parturition and assists in milking hygiene (or hygiene during nursing of kids) after kidding. Ketosis, hypocalcemia, abortion, stillbirth, and dystocia are conditions that can be expected. Prevention of postpartum complications and success of a pasteurization and pathogen-control program depend on detecting the onset of parturition and attending the birth of kids. Rapid detection and assistance with dystocia and immediate removal of kids before they nurse for pasteurized rearing programs can be aided by frequent observation of does near term. Induction of parturition with prostaglandin can be used as an aid to disease control programs that require removal of kids at birth. Some producers use infant room monitors to remotely detect the onset of second stage labor (does usually vocalize during second stage labor). Dystocia in does is usually due either to the presence of a large single fetus or to malpresentation of multiple kids that become tangled. Additionally, does that give birth to multiple kids may exhibit uterine inertia, which requires that the kids be delivered manually and that the doe be treated for hypocalcemia. Most malpresentations are easily corrected and the kids delivered. Occasionally a cesarean section is required to deliver a large kid or, more commonly, is required when the cervix fails to dilate. Natural births occur rapidly, particularly in multiparous does, which may deliver multiple kids in 30 to 60 minutes. Does attempting to deliver kids without success for more than 30 minutes of second stage labor should be examined.

POSTPARTUM CARE OF THE DOE

Does should be carefully examined for the presence of additional fetuses. The veterinarian can perform routine abdominal ballottement by standing over the doe, reaching around the caudal abdomen and joining hands, then lifting up sharply. A retained fetus may be detected as a firm mass. Ultrasonographic examination confirms this finding, but greater care must be taken to visualize the fetus once the fluid contrast is lost after the chorioallantoic membrane has ruptured. Does should also be examined for trauma or hemorrhage. Assessment of the doe's vital signs and muscle tone after parturition is useful in detecting hypocalcemia. Hypocalcemia may predispose does to uterine prolapse.

After kidding, the doe passes the placenta rapidly—often within 1 hour of the last kid's birth—but the placenta is not considered retained until 8 to 12 hours post partum. At this time, light manual traction, oxytocin (if within 48 hours post partum), and systemic or local antibiotics can be administered. In normal does, lochia can be discharged for up to 4 weeks. Lochia should be carefully cleaned from the udder before milking. Lochia from normal births may contain *Chlamydia psittaci, Coxiella burnetii,* or other pathogens that are infectious to humans and other goats. Normal reddish brown lochia must be distinguished from the brownish, watery, malodorous discharge that accompanies postpartum metritis. Does with metritis are usually febrile and partially anorectic and have depressed milk production. These does should be treated with local or systemic antibiotics and nonsteroidal anti-inflammatory drugs, and may require supportive therapy.

Dairy does should be milked soon after parturition. Even in herds in which the manager does not elect to feed heat-treated colostrum, milking the doe and hand-feeding the kids ensures maximum first feeding ingestion of colostrum by all kids. In meat and fiber production herds, does' udders should be palpated for evidence of mastitis and to evaluate sufficiency of milk production, and milk should be expressed from each teat to assess the patency of the teat and to detect abnormal secretions. Does with good milk production that give birth to a single kid should be considered as candidates for cross-fostering another kid.

In meat, fiber, or other goat herds in which does nurse their kids or otherwise are unaccustomed to frequent handling, penning the doe with her litter for several days allows close observation without having to gather does and potentially disrupting maternal bonding. This allows the caretaker to assure that both udder halves are being nursed and to monitor for the presence of mastitis and adequacy of milk production (and milk intake by her kids). This also facilitates the doe bonding with all members of the litter and aids in decision-making as to whether a doe can raise her entire litter or whether one or more kids should be hand-reared or fostered to another dam. Does and their litters may more smoothly transition into the main herd if moved from maternal pens into small group pens (with does/litters from the same kidding period) for several days before returning to the larger herd.

Postpartum does should be watched closely for signs of hypocalcemia or ketosis. Maximizing dry matter intake of fresh does will help to prevent metabolic disease and ensure maximal peak milk production. Does should be monitored for their ability to compete at feeders (and moved if needed), and fresh supplies of water and high-

quality forage should be provided immediately to encourage early return to normal feed intake.

POSTNATAL CARE OF KIDS

At the time of birth, kids should be observed for normal respiration, evidence of respiratory acidosis, and other evidence of fetal distress such as meconium staining. Mucus and fluids should be immediately removed from the nose and mouth of newborn kids. Aspiration of meconium should be suspected in kids with extensive meconium staining that demonstrate respiratory difficulty. For cases under intensive clinical management, oxygen or doxapram hydrochloride, or both, may be needed to support or stimulate respiration, especially in premature kids. Mild to moderate acidosis can be treated with intravenous HCO_3 at 1.0 mEq/kg or as determined after the base deficit is analyzed. Kids and placentas are examined for abnormalities that would suggest placentitis or other signs of in utero infection that might warrant submission for necropsy.

If the owner plans to rear kids using pasteurized colostrum methods to prevent transmission of pathogens such as caprine arthritis-encephalitis virus (CAEV) or *Mycoplasma* spp., the kids should be removed from the doe at birth, before the doe has been allowed to lick them. In meat or fiber production herds, disruption of bonding between the doe and kids should be minimized. However, the umbilicus of all kids should be inspected for hemorrhage or herniation, and the umbilical stump disinfected with tincture of iodine or chlorhexidine solution. Although chlorhexidine (1:4 dilution of concentrate) is preferred over 2% or 7% iodine for foals, similar studies have not yet been conducted in ruminants. Continued treatment of the umbilicus for several days is preferred. Kids should be examined for the presence of congenital defects such as pseudohermaphroditism, teat anomalies, cryptorchidism, atresia ani, cleft palate, brachygnathia, prognathia, and congenital goiter.[1]

In herds using pasteurized kid-rearing methods, kids are removed at birth and hand-fed heat-treated goat colostrum or cow colostrum (heat-treated preferred) by nipple bottle. A sucking reflex can be stimulated by stroking the kid's face behind its muzzle. Weak kids can be given colostrum with the use of a soft rubber catheter as a stomach tube and the barrel of a 60-ml catheter-tip syringe as a reservoir for gravity flow. Depression caused by respiratory acidosis may reduce suckling and result in decreased colostrum intake. Delayed colostrum intake, inadequate colostrum ingestion, and ingestion of poor-quality colostrum are common reasons for failure of passive transfer.

In meat and fiber production herds and other herds using dam-rearing of kids, adequate colostrum intake should be assessed. Kids nursing from a dam that has been determined to have adequate colostrum and palpation of kids' abdomens after nursing serve as indicators of colostrum consumption. However, hand-feeding of colostrum to all kids is the most definitive means of ensuring adequate colostral intake. Failure of passive transfer can be confirmed by screening serum immunoglobulins using zinc sulfate turbidity, sodium sulfite precipitation, and other screening techniques.[2] Serum immunoglobulin G levels greater than 1600 mg/dl are most desirable. Serum immunoglobulin G levels less than 600 mg/dl indicate failure of passive transfer, and partial failure of passive transfer is suggested by serum immunoglobulin G levels between 600 and 1600 mg/dl.[2] Transfusion of 20 to 40 ml/kg caprine plasma intravenously may be indicated for valuable neonatal kids with failure of passive transfer.[2]

If the use of goat colostrum is planned, or if the cow colostrum is from a predictable source, vaccination of the donor dam 1 month before parturition against *Clostridium perfringens* types C and D, tetanus, and other appropriate pathogens will maximize specific immunoglobulin concentration in colostrum. Regardless of source, colostrum must be of high immunoglobulin concentration and have good nutritional quality. Does that leaked colostrum or were milked because of premature distention of the udder will have colostrum of low immunoglobulin, vitamin, and fat content. Colostrum with an immunoglobulin content greater than 6 g/dl and specific gravity of at least 1.050 is most desirable.[2,3] Microbial contamination can be minimized by the use of hygienic milking practices. Clipping the doe's udder and thighs before parturition and cleaning the teats before milking or allowing kids to nurse will minimize bacterial contamination of colostrum and prevent ingestion of environmental organisms by kids. The bacterial counts of colostrum that is allowed to sit for long periods, especially in warm environments or where flies are present in large numbers, may exceed 250,000 colonies per milliliter. If stored, colostrum should be refrigerated or frozen in small containers to allow rapid cooling and to minimize bacterial growth.

Pasteurized-rearing programs are recommended to prevent transmission of CAEV, mycoplasma, and other pathogens. Removal of kids at birth may also reduce their likelihood of exposure to Johne's disease and other organisms. Heat treatment of colostrum for 1 hour at 56° C has been demonstrated to prevent CAEV transmission in colostrum.[4] Colostrum is heated in a double boiler to 56° C and is either held for 1 hour while being stirred or is transferred to a preheated vacuum flask for 1 hour. Monitoring the temperature of colostrum or milk at the end of the heat treatment period is critical to ensuring the success of the heat treatment. Colostrum that drops below 55° C at the end of the treatment period should be discarded or reheated. Care must be taken to avoid overheating colostrum. Heating of colostrum above 58.9° C causes denaturation of immunoglobulins and other proteins and results in clumping. Feeding heat-damaged colostrum, even if filtered, usually results in osmotic diarrhea. Heat-treating colostrum in a microwave oven is not recommended because of unequal heating properties that could result in denaturing some of the colostrum while inadequately heating other portions.

High-quality, first-milking colostrum can be heat-treated and banked for later use. Heat-treated colostrum is often frozen in small containers and thawed as needed. Frozen colostrum is best thawed in a warm water bath. Microwave thaw techniques have been used but may result in unequal heating with accompanying denatura-

tion of portions of the colostrum. Repeated freezing of thawed colostrum and storage of frozen colostrum for longer than 1 year are not recommended.

Cow colostrum can be used instead of goat colostrum; however, goat owners must take steps to ensure that the colostrum quality and freedom from *Mycobacterium paratuberculosis* or other enteric pathogens meet the same standards that they would demand from goat colostrum. Neonatal isoerythrolysis has been reported following ingestion of cow colostrum, but appears to be quite rare. Artificial colostrum substitutes have been used with mixed results. Higher serum immunoglobulin G levels are obtained in kids after ingestion of goat or cow colostrum than after colostrum substitutes.[5,6] Colostrum substitutes prepared from concentrated serum immunoglobulins appear to have more promise than those prepared from whey concentrate.

After the initial colostrum feeding, kids can be fed pasteurized milk or additional heat-treated colostrum if available. Milk for pasteurized-reared kids should be heated to a minimum of 73.9°C for 15 seconds to destroy *Coxiella burnetii*. Lower temperature and longer time schemes control other pathogens. Home pasteurization devices are widely available, but producers must monitor the exit temperatures frequently to ensure proper adjustment of the pasteurizer unit. Also, a system for identification of pasteurized milk—for example, using food coloring—may aid in avoiding the accidental feeding of raw milk. Assignment of a single person to feed kids helps to minimize errors in a pasteurized-rearing program.

Large cardboard boxes with clean bedding material work well for housing newborn dairy goat kids, especially in large herds. A doe's kids can be placed in one box, and the dam's identification written on the box as a means of identifying kids until they can be labeled with paper collars and permanently identified by tattoo. Disposable boxes are a useful means of preventing build-up and spread of enteric pathogens. Kids can be kept in these boxes for about 2 weeks, after which the box can be destroyed and kids housed in larger groups.

In herds in which kids are dam-reared, the doe and kids must have access to draft-free shelter from environmental extremes to minimize risk of hypothermia, heat-stress, or pneumonia. Young kids naturally seek out protected "hiding" places where they spend many hours sleeping away from their dam, with the dam returning to nurse her kids for brief periods. Herd owners can provide small boxes in pastures for kids to use. Restricting does to a small pasture or paddock during kidding and for a few days may be helpful in ensuring strong maternal bonding. Regardless of rearing method, kids must be raised in a clean, uncrowded environment.

Dairy kids are generally fed from nipple bottles or buckets with multiple nipples. Weaning is recommended at 2 to 3 months of age. Dam-reared kids raised for meat can be weaned at a later age. Pasteurized goat milk or cow milk should be fed for at least the first 2 to 4 weeks of life. The use of milk replacers for growing kids has met with mixed success. High-fat, low-lactose, high-protein, and low-fiber milk replacers are preferred. High-lactose milk replacers in particular may be associated with digestive upsets, including bloating and death. High-quality calf and lamb milk replacers, and some goat milk replacers, have been used successfully by producers. Mixing milk replacers (one half reconstituted milk replacer, one half whole milk) seems to minimize problems associated with milk replacers and increases their palatability. Further information on kid nutrition and management is available elsewhere.[7,8]

Horned dairy kids should be disbudded as soon as the horn bud can be distinctly palpated, usually at 5 to 7 days of age. Swiss breed kids may have palpable horn buds at a few days of age, while Nubian kids may take up to 2 weeks to develop distinctly palpable horn buds. Identification of the characteristic swirls of hair and visualization of the differentiated germinal epithelium of the horn bud before disbudding will help to avoid accidental "disbudding" of naturally polled kids. Early disbudding of kids with a disbudding iron is much less stressful and far more humane than surgical removal of scurs or horns at a later age.

During the first few days of life, kids are at greatest risk of hypothermia, hypoglycemia, and colibacillosis. Hypoglycemic kids can be treated with intraperitoneal administration of up to 50 ml of warmed 10% to 25% glucose solution, diluted with up to 100 ml isotonic sodium chloride or lactated Ringer's solution. Cryptosporidiosis and floppy kid syndrome are most commonly seen in kids from 3 to 10 days of age. Clinical signs of white muscle disease, copper deficiency, *Pasteurella haemolytica* pneumonia, mycoplasmosis, and coccidiosis frequently begin to occur at between 2 and 4 weeks of age. Additional information on kid diseases is available elsewhere.[7,8]

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CHAPTER 75

Puerperal Nutrition and Metabolic Diseases

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The time around parturition is the single most stressful event in a doe's reproductive life cycle. Metabolic and nutritional insults before and following parturition can result in exacerbation of key metabolic changes necessary to make the physiologic transition from pregnancy to lactation, resulting in a myriad of metabolic diseases. Current perceptions tend to focus attention on the lactating doe and nutritional support of milk production; however, nutrition and management of the late pregnant doe has more significant impact on her productive efficiency, both reproductive and lactational. The focus of this chapter will be to highlight the impact of prepartum nutrition and management on periparturient metabolic disease and reproduction.

METABOLIC ADAPTATIONS AROUND KIDDING

An appreciation of the exquisite metabolic adaptations the doe must undergo to achieve a successful transition from pregnancy into lactation is key to understanding the critical role of nutrition on metabolic disease and reproductive performance. Minimal data are available regarding pregnant doe metabolism and nutrition. Given the similarity in metabolic responses observed with dairy cattle and sheep, current research concepts regarding physiologic alterations associated with the transition from pregnancy to lactation will be extrapolated from these species to the pregnant doe. Fetal metabolism and maternal metabolic adaptations over the periparturient period were detailed in Chapter 58. Important concepts as they relate to induction of metabolic disease or influencing reproductive performance are summarized here.

Fetal Metabolism

Metabolic activity of the fetus is nearly twice that of the dam, and fetus, placenta, and uterus combined (conceptus) consume nearly 50% and 80% of maternal glucose and amino acid supply, respectively.[1] The conceptus oxidizes glucose and its placental derivative, lactate, as its primary metabolic fuel; however, glucose and lactate oxidation only account for 50% to 60% of fetal caloric needs.[2] Amino acids, even in a well-fed dam, account for 30% to 40% of total conceptus caloric requirement.[2] Fetal utilization of glucose is dependent upon maternal glucose availability and with maternal undernutrition, utilization is markedly decreased. Amino acid delivery to the conceptus is independent of maternal dietary adequacy, and thus, amino acids become a primary fetal energy substance when glucose is unavailable.[3] Placental active transport and catabolism of maternal skeletal protein account for the ability of the dam to maintain consistent amino acid delivery to the conceptus.[1,2] In spite of their ready availability in maternal circulation, nonesterified fatty acids (NEFAs) and ketone bodies are not significant energy substrates for the gravid uterus as they do not efficiently cross the placenta.[2] This metabolic milieu provides a buffering system to maintain fetal growth in the face of inadequate maternal nutrition, yet not facilitate excessive fetal growth at the expense of maternal energy reserves (fat).

All macro- and microminerals are efficiently transferred from maternal circulation to the conceptus. Within the fetus, minerals are concentrated in the fetal liver as well as being utilized to meet their specific metabolic or structural physiologic roles.[4] Concentration of mineral elements within the fetal liver provides a mineral reserve in support of the neonate's higher metabolic rate and in the face of consuming a trace mineral–deficient diet (e.g., milk). Fat-soluble vitamins A, D, and E do not efficiently cross the placenta, but are concentrated in colostrum.[5–7] With the transfer of minerals and vitamins from maternal circulation to fetus and colostrum, adequacy of the late gestation maternal diet will ultimately dictate nutrient status of dam and neonate and mediate disease susceptibility potential, especially susceptibility to infectious diseases, given the important role trace minerals and fat-soluble vitamins play in immune function.

Maternal Adaptations

The goal of maternal metabolic adaptations is to increase glucose availability in support of pregnancy or subsequently lactation. To meet glucose needs during the transition from late pregnancy to lactation, maternal tissues reduce their use of glucose as an energy source, increase rate of hepatic gluconeogenesis, and provide sufficient endogenous glucogenic substrate (i.e., amino acids, glycerol, lactate) to account for lower nutrient intake.[8] Glucose is replaced by fatty acids and their derivative, ketone bodies, as alternative maternal metabolic fuels. To facilitate fatty acid availability, adipose tissue becomes sensitized to signals mediating reduced lipogenesis and increased lipolysis, hence increasing blood NEFA concentration. Reduced glucose uptake and increased NEFA

oxidation by skeletal muscle accounts for a significant savings of glucose; however, skeletal muscle also contributes amino acids in support of gluconeogenesis. Coordinated adaptation of adipose tissue, skeletal muscle, and liver function is mediated through changes in insulin concentration and tissue insulin resistance.[8]

Elevation of blood NEFA concentration metabolically is a double-edged sword. As described, elevation of blood NEFA in late pregnancy provides an alternative fuel for maternal nonuterine tissues. However, the liver absorbs NEFAs in direct proportion to their blood concentration. Hepatocytes have limited ability to completely oxidize influx of NEFAs, and they are partially oxidized into ketone bodies, which can be metabolized for energy by other nonuterine maternal tissues.[9] Available NEFAs can also be re-esterified into triacylglycerol (TAG) and packaged into very low density lipoprotein (VLDL) particles and exported from the liver.[9] If not exported in VLDL, then TAG is packaged into hepatocytes, resulting in increasing amounts of fatty infiltration. Ruminant animals in general are not efficient at exporting VLDL particles[10] and thus experience variable degrees of hepatic fatty infiltration around the time of parturition.[9]

Similar to cattle and sheep, blood NEFA concentration in goats is a metabolic marker of energy balance and degree of fat mobilization.[11] Excessive elevation of blood NEFA concentration can result from factors inducing a stress response (β-adrenergic agents stimulate lipolysis), prolonged or severe negative energy balance, or both. Dry matter intake typically declines just before, and slowly increases following parturition, placing the animal in a state of negative energy balance. Errors in feeding management, poor quality feeds, adverse environmental conditions, and number of fetuses, among other factors, can exacerbate severity and duration of negative energy balance. Hepatic uptake of blood NEFA beyond its metabolic capacity to process will result in maladaptive responses leading to ketosis and increasing fatty infiltration, which compromise hepatic function and initiate a deteriorating metabolic cycle of hypoglycemia, fat mobilization, and severe fatty infiltration.

Although hypocalcemia is not as prevalent in goats compared to dairy cattle, the doe's calcium homeostatic system must adapt to the challenges of calcium outflows associated with fetal growth and initiation of lactation. Limited studies have specifically addressed regulation of calcium metabolism in the goat, but evidence suggests similar controlling mechanisms compared to cattle.[12] Maternal adaptations to trace mineral and fat-soluble vitamin losses to fetus(es) and colostrum are limited to mobilization of reserves and replenishment from adequate dietary supplementation.

PREPARTUM NUTRITION AND REPRODUCTION

Nutrient requirements of the late pregnant, nonlactating doe are only slightly higher than maintenance, approximately equivalent to the energy and protein required to produce 2 to 4 lb of 4% milk per day.[13] However, providing sufficient quantities of essential nutrients is just as critical for the late pregnant doe as the lactating doe to maintain optimal performance. Recognition of a substantial increase in nutrient requirements for the late pregnant and lactating animal compared to maintenance has been the focus of nutritional investigations in dairy cattle and ewes. Dietary recommendations for digestible energy (DE), crude protein (CP), calcium (Ca), and phosphorus (P) for the late gestation doe are 1.5 to 1.8 times greater compared to maintenance at the same level of activity.[13] The transition from late gestation into lactation requires a similar or much greater increase in dietary nutrient intake. These differences in nutrient requirements require appropriate modifications in the feeding program as well as metabolic alterations by the doe to adequately support late gestation and lactation. If these metabolic changes are not effectively enacted, metabolic disease, reduced milk production, and impaired reproductive performance may result.

Kid Viability and Survival

Data from cattle and sheep suggest that nutrition of the dam at all stages of gestation can influence neonate viability and productivity. In reviewing factors responsible for contributing to prepartum and partum calf[14] or lamb[15,16] losses, nutritional deficiencies and toxicities influenced all factors. Similar contributing factors can be reasonably assumed for goats. Maternal undernutrition during mid- to late gestation has been implicated in an abortion syndrome observed in yearling Angora goats.[17]

Birth weight is the single most important factor determining postnatal survival. Extremely heavy birth weight is more associated with dystocia, and lighter birth weight kids, typical of twins and triplets, have higher mortality rates.[18] Dynamic in vivo measures of fetal sheep crown-to-rump length found fetal growth to be deterred or completely stopped during periods of induced maternal hypoglycemia during late pregnancy.[19] Ditocous ewes fed an 8% CP diet gave birth to lambs that were 20% lighter than lambs born to similar ewes fed isocaloric diets with either 11% or 15% CP.[20] Ewes fed 11% CP diets, however, mobilized maternal protein in support of fetal growth, whereas maternal protein accretion occurred in ewes fed 15% CP diets. In contrast, singleton-bearing ewes fed 1.4 times their estimated protein requirement (165 g CP; 11.8% CP) delivered larger lambs (4.9 versus 4.3 kg) with greater lambing difficulty and higher mortality rate compared to ewes fed to requirement (117 g CP; 8.7% CP).[21] Besides differing in using twin or singleton pregnant ewes, dietary treatments were initiated at 110 days[20] and 85 days[21] of gestation for these two studies.

Maternal dietary influence on fetal growth is more complicated than simply addressing under- or overfeeding relative to requirement. Maternal body condition score and dietary nutrient status relative to period of fetal and placental growth are confounding variables.[1] Fat ewes partition more nutrients to the gravid uterus, maintaining fetal growth during periods of moderate undernutrition in late pregnancy compared to lean ewes.[22] Lean or moderately fat ewes fed ad libitum in late pregnancy had similar placental and fetal birth weights despite different intake amounts (29% higher for lean ewes), suggesting placental mitigation of available nutrients in

controlling fetal growth.[23] West African dwarf does fed a higher level of concentrate throughout gestation had heavier birth weight kids and greater problems with dystocia, whereas does fed the same rate of concentrate for only the third trimester (>120 days) had low birth weight kids with higher mortality rate.[24] Does fed a higher and moderate amount of concentrates in second (60 to 120 days) and third trimester, respectively, had moderate birth weights and minimal health problems. High rate of energy supplementation during first and third trimester in does was suggested to be avoided.[25] Twin-bearing Tswana does supplemented throughout pregnancy had greater total kid birth weight compared to does not supplemented or supplemented before or after 103 days of gestation.[26] Although placental mass was not influenced by dietary treatment, nonsupplemented does had the lowest placental (390 versus 580 g) and total fetal (5.1 versus 5.8 kg) weights.[26]

In primigravid, singleton-bearing ewes, placental growth and, ultimately, lamb birth weight were restricted when fed for rapid growth after the first trimester.[27] Rapid maternal growth during the first trimester followed by moderate growth stimulated compensatory placental growth and moderate birth weight lamb.[27] Placental weight is the primary determinant of fetal weight.[28] Fetal cotyledon number was influenced by first trimester nutritional status, whereas cotyledon weight was mediated by second trimester nutrition.[27] Fetal number and placement within uterine horns further mitigate the relationship between gestational nutrition and fetal growth.[28]

Beyond birth weight, maternal milk production will affect growth and survival of the neonate. Inadequate nutrition during late pregnancy influences milk production and composition,[29] possibly as a result of compromised mammary gland development.[30] Dietary protein content of 11% CP (9.8 g/kg body weight × 0.75), slightly higher than the National Research Council (NRC) recommendations,[13] is recommended for adequate late gestation nutrition to meet fetal and subsequent lactational needs.[31] Current NRC pregnancy energy requirements were considered adequate;[29] however, a new NRC publication for goat nutrient requirements is under development.

Colostrum Quality and Quantity

Following birth weight concerns, adequate consumption of good quality colostrum is essential to neonatal survival. With selection for higher milk production in dairy goat breeds, potential exists for selecting against colostrum quality, as a result of increased colostrum volume, similar to what has been experienced in the dairy industry. Limited studies, with equivocal results, have addressed nutritional effects on colostrum quality in sheep and goats. Contrary to what might be expected, feeding greater amounts of prepartum protein (117 versus 165 g/day) resulted in reduced colostrum yield in sheep, but immunoglobulin concentration was not reported.[21] Energy and protein content of the prepartum diet for pregnant ewes influenced the amount of colostrum produced at 3 and 24 hours after lambing.[32] Colostrum volume increased with increasing supply of energy and protein (Table 75-1). In ewes, increasing prepartum energy intake increased colostrum yield, and increasing prepartum protein intake improved lamb colostral immunoglobin absorption efficiency.[33] Although feeding supplemental concentrate increased colostrum yield, immunoglobulin concentration was decreased even with increasing undegradable protein supplementation.[34] It would seem that beyond severe undernutrition, colostrum quality is not greatly influenced by additional energy or protein supplementation and may adversely affect quality by increasing volume. Further work is needed to fully explore prepartum diet effects on colostrum production and quality.

Table **75-1**

Effect of Prepartum Dietary Metabolizable Energy (ME) and Crude Protein (CP) Intake on Colostrum Production in Sheep

	Low Energy		High Energy	
ME intake, Mcal/d	1.94	1.94	3.46	3.46
CP intake, g/d	80	128	128	185
ME:CP	24	15	27	19
	Colostrum Volume, ml			
First 3 hours	150	320	380	640
24 hours	1020	1580	1890	2100

Adapted from Robinson JJ: Energy and protein requirements of the ewe. In Haresign W, Cole DJA (eds): *Recent advances in animal nutrition.* London: Butterworths: 1988, pp 187–204.

Fetal Reproductive Development

Adequacy of the prepartum diet has been shown to influence embryonic and fetal ovarian development and subsequent reproductive performance in the adult ewe.[35] Gunn and colleagues[36] observed a reduction in lambing percentage from ewe lambs born to ewes exposed to undernutrition during their fetal development compared to ewe lambs born to well-fed pregnant ewes. Increased numbers of high-quality embryos were collected from lambs born to ewes fed a higher level of nutrition (1.5 times maintenance) during mid-gestation (71–100 days), late gestation (101–126 days), or both.[37] Whether this nutritional effect is influencing ovarian growth, follicular development, or embryo survival is not well defined, but given the lifetime ramifications, further study to elucidate mechanisms is warranted.

Periparturient Disease

Unlike the situation for cattle given the difference in time frame between parturition and subsequent mating, periparturient disease of goats plays a lesser role in mitigating reproductive performance than recognized for dairy cattle. If the periparturient disease process induces severe body condition loss and an inability to regain condition before breeding, then reproductive performance may be adversely affected. The most significant reproductive effect of periparturient disease is reduced survival of off-

spring and dam rather than specific impact on subsequent reproductive efficiency. Role of prepartum nutrition in the pathogenesis of periparturient diseases is discussed in the following sections.

DERANGEMENTS OF ENERGY BALANCE (PREGNANCY TOXEMIA, KETOSIS, AND FATTY LIVER SYNDROME)

An inability of the pregnant or lactating doe to maintain glucose homeostasis characterizes a state of negative energy balance whereby glucogenic precursors from diet or endogenous sources are insufficient to meet the doe's total energy requirement. Metabolic signals recognizing glucose insufficiency initiate fat mobilization in proportion to degree of negative energy balance to compensate. If magnitude or duration of the negative energy balance insult is severe enough, release of NEFA may overwhelm the liver's capacity, culminating in maladaptive metabolic responses generating large quantities of ketone bodies and allowing severe fatty infiltration of hepatocytes. Ability to maintain functional insulin concentration and maternal tissue responsiveness seems pivotal to appropriate metabolic adaptation to negative energy balance.[9,38] Inherent metabolic differences among individuals (genetic variation) may account for observed disparity in individual susceptibility to clinical manifestations.[39]

Pregnancy ketosis (pregnancy toxemia) can occur during the last 6 weeks of pregnancy, concurrent with the period of rapid fetal growth, although it is most often recognized in the last 2 to 4 weeks of gestation. The last few weeks of gestation are often associated with declining intake, and when in combination with a pregnancy of multiple fetuses, place the doe in a precarious ketosis-prone metabolic position energetically. Lactational ketosis occurs most frequently within the first 4 weeks of lactation, a period associated with rapidly increasing milk production with slowly increasing feed intake. Pregnancy toxemia is the more common problem seen in goats; however, lactational ketosis is of concern with high-producing dairy goat breeds.

Any animal, management, or environmental factors that adversely affect the doe's net energy intake increases the risk of metabolic maladaptation leading to ketosis and fatty liver infiltration. Overconditioned does (>4.0 body condition score [1 to 5 scale]) are at high risk for ketosis and fatty liver as they experience reduced feed intake compared to leaner does and have abundant fat reserves to mobilize. Feed availability and quality, sudden feed changes, water availability, transportation of late gravid does (>100 days), concurrent disease, and heat or cold stress conditions are some of the factors that, singly or in combination, may initiate an episode of reduced feed intake potentially leading to a ketotic response.[39,40] Pregnancy toxemia or lactational ketosis induced by a concurrent disease is often categorized as secondary ketosis, as typically the underlying disease was responsible for reduced intake and inducing the ketotic state.

Most often, pregnancy toxemia or lactational ketosis is a sporadic occurrence of low morbidity (<3%) within a goat herd. Individual variation in the metabolic response to herd nutrition and management accounts for the sporadic nature of the disease. In contrast, factors influencing a large percentage of the herd, namely nutritional and feeding management factors, can result in a herd outbreak with morbidity rates exceeding 10% of the does. Timely recognition of individual cases and underlying etiology are important in heading off potential herd problems as mortality rates often exceed 80% in affected animals.[39]

Clinical Signs and Diagnosis

Clinical presentation of an affected doe will vary with associated metabolic derangements. Initially with pregnancy toxemia clinical signs will be vague, primarily behavioral and attitude changes. Does will generally isolate themselves and appear listless, dull, and depressed. As hypoglycemia and ketonemia progress, neurologic manifestations become evident. Does may appear blind, become disoriented and ataxic, and have reduced feed intake. Metabolic acidosis develops from excessive ketone body production. Breathing may become more rapid, neurologic signs continue to progress, and the doe is less likely to rise or respond to stimuli. Chewing, teeth grinding, and vigorous licking movements may be seen. In the final stages the doe becomes recumbent with severe neurologic signs and coma. Death is usually the result of renal failure or toxemia following fetal death. Does may show slight signs of recovery following fetal death but may ultimately succumb as a result of toxin release. Progression of clinical signs ranges from 12 hours to 7 days, a more typical time course being 3 to 4 days.[39,40] Differential diagnoses should include hypocalcemia, polioencephalomalacia, listeriosis, and ruminal acidosis (grain overload).

Lactational ketosis does may misbehave in the milking parlor, exhibiting irritability; have a poor appetite; or intermittently consume feed, especially concentrates. The most obvious sign is markedly reduced milk production and excessive weight loss. Clinical signs generally do not progress like that observed in pregnancy toxemia, as the reduction in milk production reduces negative energy balance and mitigates disease progression. More often milkers readily recognize reduced milk production and initiate treatment.

Ketosis is definitively diagnosed by identifying excessive positive ketone reaction in urine. Dipsticks and ketone powder both use the nitroprusside test to semi-quantify acetoacetate concentration. Serum or plasma β-hydroxybutyrate (BHB) concentrations can also be determined for diagnosis. Expected BHB concentration is below 8 mg/dl (0.76 mmol/L), whereas concentrations above 15 mg/dl (1.4 mmol/L) indicate moderate ketogenesis and subclinical disease. Clinical ketosis is often associated with BHB concentrations in excess of 25 mg/dl (2.4 mmol/L) and may exceed 40 mg/dl (3.8 mmol/L). Other laboratory findings suggestive of ketosis include elevated NEFA (>0.4 mEq/L) and hypoglycemia (<30 mg/dl). Depending upon the degree of associated fatty liver, elevation of some liver enzymes and lower total cholesterol concentration may be present. Degree of fatty liver infiltration is most often diagnosed on necropsy. In the

later stage of the disease, hyperglycemia, hypokalemia, hypocalcemia, and elevated creatinine and urea nitrogen may be evident. Hyperglycemia may occur following fetal death. Anorexia may account for hypokalemia and hypocalcemia. Associated dehydration results in elevated creatinine and urea nitrogen concentrations.

Treatment

Treatment and prognosis for recovery of pregnancy ketosis is dependent upon the stage of the disease when recognized and treatment initiated. If the doe is still responsive and willing to eat, dietary modification and supplementation with glucogenic precursors may be adequate. Ensure that high-quality forage is provided and consumed and fermentable energy sources (cereal grains) are appropriately supplemented. Potential glucogenic precursors to be administered orally might include propylene glycol (60 ml twice daily), glycerol (45–60 ml twice daily), or calcium propionate (40–50 g diluted in water and tubed for up to three treatments).[39–41] A number of commercial products based on these glucogenic compounds are now available. Alternatively, use of oral calf scours products containing electrolytes, glucose (40–55 g), and glycine have been administered orally every 4 to 8 hours.[39,42] If the doe is in a more advanced stage of the disease, more aggressive therapy is needed and prognosis becomes progressively guarded. Given economic issues, owners need to be well appraised of the situation and potential outcome before initiating therapy.

Hypoglycemia should be corrected with the administration of glucose via intravenous or oral administration. Bolus intravenous glucose (50 g) can be administered once daily or distributed out in multiple treatments (10 g every 4 hours) depending upon practicality, owner capabilities, and economic considerations.[42] For valuable animals, a constant 5% dextrose infusion may be used. The doe's glucose status before administering therapy should be evaluated, as late-stage disease is often associated with hyperglycemia.[42] Additionally, insulin (10–25 U Ultralente subcutaneously every other day for three treatments) can be administered along with dextrose to facilitate tissue glucose uptake and inhibit fatty acid mobilization.[39,42] Acid-base status, degree of dehydration, associated electrolyte abnormalities (typically hypokalemia and hypocalcemia), and renal function should be evaluated and appropriately balanced fluid therapy administered.[40,41] Fluids with electrolytes can be therapeutically effective either intravenously or orally, depending upon the doe's status. Hypocalcemia is often associated with pregnancy toxemia and has been implicated in blunting endogenous glucose production.[43] Crystalline amino acid solutions (8.5%, 0.6–1.2 g/kg body weight) can be combined with intravenous replacement fluids. Amino acid supplementation may provide additional glucogenic substrate and may be beneficial in augmenting hepatic synthesis and export of VLDL, thus minimizing further hepatic fatty infiltration.[44]

To reduce glucose demand and improve potential response to therapy, induction of parturition is recommended if the doe is less than 2 weeks from her due date. Continued metabolic derangement is as harmful to unborn kids as is the risk of premature birth. Does that experience pregnancy toxemia and do not succumb are at higher risk for dystocia and kid death.[40] Real-time ultrasonography can be used to evaluate fetal viability in making a decision on fetal removal. Parturition can be induced with prostaglandin injection, and does can be pretreated with dexamethasone to facilitate fetal lung development if the due date is uncertain.[41] Does that fail to kid within 72 hours after parturition induction should undergo cesarean section unless they are eating well and fetal viability is confirmed by ultrasonographic examination. Does that are down, are obtunded, have neurologic signs, or have renal failure have a very poor prognosis for recovery, and fetal viability is likely compromised.

Beyond initial therapeutic procedures, ketosis and fatty liver cases can benefit from various supportive therapy modalities. With various degrees of inappetence a common presentation, stimulation of appetite is critical. Often B-complex vitamin solutions, or more specifically, vitamin B_{12}, are administered to nonspecifically stimulate appetite. Offering fresh cut grasses or a variety of feeds may help stimulate intake in some goats. If rumen function is compromised, rumen transfaunation may be required to stimulate intake. Alfalfa meal and commercial concentrate pellets can be soaked and administered through a stomach tube as gruel to provide supportive enteral nutrition.

Lactation ketosis can be treated with any one or a combination of intravenous glucose (250–400 ml, 10% solution), oral glucose (oral calf electrolyte solutions), or propylene glycol (30–60 ml twice daily). Often one or two treatments are adequate as long as an acceptable diet is available and consumed by the doe. Glucocorticoid therapy should be used cautiously if there is evidence of an infectious process. Failure to respond to treatment requires does be examined for possible concurrent disease.

Prevention

Ketosis and fatty liver are best prevented by proper nutrition and management during late pregnancy. Late pregnancy diets should be properly balanced to provide adequate rumen-fermentable carbohydrate and degradable protein to support rumen microbial growth. Current recommendations suggest a minimum of 11% CP in the late pregnant diet.[31] Fermentable carbohydrate sources, primarily cereal grains (a minimum of 0.5 to 1 lb per day up to 2 lb per day), should be increased to meet energy needs, based on fetal numbers. Sufficient effective fiber needs to be consumed to meet the minimal rumen microbial needs. Goats, being highly selective eaters, often can select out poorer quality stems of alfalfa and not receive sufficient dietary effective fiber. Wheat straw, bean straw, beet or citrus pulp, wheat middlings, or wheat bran are suitable fiber sources. Ideally, late pregnant does should be identified by number of fetuses and fed an appropriate diet. If fetal numbers are not known, then diets sufficient to support twins should be fed.

A primary management concern in preventing ketosis problems is body condition of the does. Overconditioned does are often the result of overfeeding during the dry

period when they are maintained in their normal cohort because of social problems encountered in regrouping. Body condition should be monitored and appropriate dietary changes made to ensure all does maintain an appropriate amount of body condition throughout late pregnancy. Other management issues such as feed bunk space, feed availability or abrupt changes in diet, transport, or weather-related stressors must be addressed.

Based on dairy cattle research, feed supplements of niacin (1–2 g) or rumen-protected choline (2–5 g choline) can provide lipotrophic effects in helping the liver better process fatty acids as well as reduce fat mobilization. Dairy cattle studies have shown a reduction in ketosis prevalence, lower NEFA concentration at parturition, and reduced liver fat content when supplemented before and following parturition. However, most positive responses were reported in studies in which the cows were of high body condition scores (>4.0/5.0). Evidence is lacking to support the use of these supplements in a therapeutic, rather than a preventive, mode.

DERANGEMENT OF CALCIUM HOMEOSTASIS (HYPOCALCEMIA, MILK FEVER)

Dairy goats may develop hypocalcemia in late gestation, around the time of parturition, or during early lactation.[45,46] High-producing, older (>3 years) dairy does are more predisposed to hypocalcemia following kidding, similar to the syndrome observed in dairy cattle. However, most goats have a relatively large fetoplacental unit, which predisposes them to development of prepartum hypocalcemia. Although often discussed as an important clinical concern, prevalence of hypocalcemia in goats is much lower than the incidence observed in dairy cattle. A common presentation of hypocalcemia in goats is secondary to pregnancy toxemia or other disease process that markedly reduces feed intake.

Hypocalcemia results from the inability of the calcium homeostatic system to maintain blood Ca in the face of Ca outflow to fetus, colostrum, or milk. Parathormone, calcitonin, and vitamin D are the primary mediators of Ca homeostasis in the goat, similar to other species.[12] Parathormone responds to low blood Ca^{2+} by stimulating activation of vitamin D, which facilitates intestinal Ca absorption efficiency, and promoting Ca resorption from bone and reabsorption from renal tubules. Calcitonin has opposing effects to parathormone, resulting in reduction of blood Ca^{2+} concentration. Pathogenesis of primary hypocalcemia in sheep and goats is not well defined, but most likely follows that of dairy cattle when sensitivity of target organs to parathormone stimulation is blunted.[47] High prepartum dietary Ca has previously been implicated, but research with dairy cattle suggests that dietary effects on acid-base status, defined as dietary cation-anion difference (DCAD), is the primary inciting problem.[47]

Clinical Signs and Diagnosis

Initially the doe is ataxic, nervous, and hyperactive. The doe is hyperirritable and may show fine muscle twitching of the lips, eyelids, and ears. Trembling or twitching of other muscles of the body may also occur. Convulsions may develop. As hypocalcemia progresses, clinical signs may include anorexia, ruminal stasis, constipation, cold ears and extremities, and flaccid paralysis. The doe quickly becomes sternally recumbent and laterally recumbent in the final stages. The head may be turned back to the flank. Body temperature usually remains normal, but will decline to subnormal in a short period of time.[46] Heart sounds are muffled with tachycardia and weak pulse. Death follows bloat, regurgitation of rumen contents, and aspiration. Less severely affected does (subclinical hypocalcemia) show lethargy, poor appetite, and uterine inertia or poor milk production, depending upon time frame of presentation.

The disease course can be as short as a few hours or can occur over a couple of days. Occasionally it may occur as "sudden death". Diagnosis is made based on history, presenting clinical signs, and rapid response to therapy. Diagnosis confirmation is accomplished by determining blood Ca concentration. Serum total Ca concentrations are decreased, usually to less than 6 mg/dl (normal, 8–12 mg/dl). More severe clinical signs are correlated with lower serum total Ca concentrations (<4.0 mg/dl). Hypophosphatemia and either hypermagnesemia or hypomagnesemia often occur concurrently.[46] To help in diagnosing hypocalcemia in a sudden death case, fluid from eye chambers obtained during a postmortem examination can be analyzed for Ca concentration up to 48 hours after death. Hypocalcemia may look like other diseases and must be differentiated from polioencephalomalacia, advanced grain overload, toxic mastitis, lead poisoning, and listeriosis.

Treatment

Recommended treatment for hypocalcemia is slow intravenous administration of 50 to 100 ml of Ca gluconate (9.3% Ca) or borogluconate (8.3% Ca) solution to effect.[46] A suggested starting dosage is 1 g Ca^{2+} per 45 kg body weight.[48] Cardiac rate and rhythm should be monitored during Ca infusion. Initially, poor peripheral perfusion limits absorption of subcutaneous Ca solutions, but it can be used following intravenous treatment to help prevent relapse (20–60 ml, 20 ml/site). Soft tissue edema occasionally occurs following subcutaneous administration of Ca solutions, especially those containing dextrose. Caution should be exercised in administering both subcutaneous and intravenous Ca solutions together, as the total dose may reach a toxic level.[48] Response to intravenous treatment should be dramatic, with the doe usually starting to shiver and brighten up by the time treatment is completed. Early treatment usually results in positive responses, and does not treated will die. A rapid progression to an obtunded state is indicative of a poor prognosis. Oral calcium gel preparations designed to correct hypocalcemia in dairy cattle can be used in goats at an appropriate body weight–based dose. However, many of these gel products are caustic and can induce pharyngeal or esophageal lesions. Following intravenous or subcutaneous injections, dietary intake of Ca should be increased with use of alfalfa hay or Ca-based mineral

supplements. Does in late gestation with hypocalcemia should be evaluated or monitored for pregnancy ketosis and treated accordingly.

Prevention

Without good scientific evidence describing the mechanism responsible for hypocalcemia in dairy goats, it is difficult to define specific feeding recommendations. It would seem prudent to maintain appropriate dietary Ca and P content in late pregnancy to support fetal bone development, but not to supplement to excess. Dietary potassium (K) should be monitored in an attempt to maintain a level below 2%. Dietary magnesium (Mg) should also be monitored and maintained according to dietary K (see next section on hypomagnesemia). Once into lactation, dietary Ca and P content should be increased to a level to support milk production capacity, which can be achieved by feeding alfalfa or other legume hay. Grass forages contain moderate to low Ca content and cereal crop forages such as wheat or oat hay are very low in Ca. If these feeds make up the forage portion of the diet, additional Ca sources, primarily from mineral supplements, should be provided.

Use of anionic salts (minerals high in chloride and sulfur) has been advocated for dairy cattle in preventing milk fever.[47] For anionic diets to be effective, they need to be properly balanced relative to dietary sodium (Na), K, chloride (Cl), and sulfur (S) to achieve a cation-anion difference ([Na + K] – [Cl – S], all values in mEq) of –10 to –15 mEq/100 g diet dry matter. In using anionic salts, the goal is to induce a state of compensated metabolic acidosis, which stimulates Ca absorption and mobilization. Work with sheep[49,50] and goats[51] has shown a similar dose-response effect of DCAD on Ca homeostasis, suggesting possible applicability. To achieve the desired effect, one must ensure the animal is appropriately acidified. This requires specialized feed ingredients to be fed and close monitoring as well as controlling dietary K content. Use of buffers such as sodium bicarbonate may increase susceptibility to hypocalcemia by contributing to diet alkalinity. For this reason, buffers are best not fed during the dry period. Urine pH measurements can be used to monitor effectiveness and titrate the required amount of anionic supplement.

In general, anionic salt feeds are not very palatable and can reduce feed intake. This is an undesirable effect, but newer, more palatable products are now available. Anionic salt products need only to be fed for a brief period of 10 to 14 days immediately before parturition to prevent hypocalcemia.

HYPOMAGNESEMIA (MILK TETANY, GRASS TETANY)

Magnesium is inefficiently absorbed from the rumen, and high dietary K content can interfere with Mg absorption. Potassium poisons the ruminal Na-K ATPase-mediated active transport system for Mg.[53] Magnesium also plays a role in maintenance of blood Ca concentrations, and hypomagnesemia can induce hypocalcemia.[47] Besides mineral interactions, differences exist between grasses and legumes as to Mg content. Grasses contain less Mg than legumes and when growing rapidly in cooler conditions (lush spring pasture), Mg availability is greatly reduced. Sheep and goats, like other ruminants, have little ability to manage blood Mg concentrations if dietary or absorption levels are depressed. The combination of low intake coupled with greater losses during early lactation result in the clinical syndrome.

Hypomagnesemia is a common problem in beef cattle on spring pasture, but sporadically is seen in dairy cattle and small ruminants. Many clinical syndromes have been identified relative to disease circumstances, but all have hypomagnesemia in common. Lactating does on spring pasture are susceptible (grass tetany or lactation tetany) as well as growing kids on milk replacer (milk tetany).[46] Animals fed low-quality winter forages that are also low in Mg content can have chronic forms of hypomagnesemia, and any stressors will precipitate a tetanic syndrome (winter tetany).

Clinical Signs and Diagnosis

Hypomagnesemia (<1.2 mg/dl) usually occurs within the first 2 to 4 weeks of lactation and results in a life-threatening disease process characterized by severe tetanic muscle spasms. Some affected animals may be found dead in the pasture. Initial clinical signs include ataxia, muscle stiffness, and hyperexcitability. All muscles are overstimulated, resulting in extreme leg stiffness and observed muscle spasms. This is very different from the paralytic muscle weakness of hypocalcemia. With declining serum Mg concentration, clinical signs progress into recumbency and paddling. Convulsions may be triggered by some stimuli including predator attacks, severe weather changes, transportation, and other stressors.

Similar to hypocalcemia, diagnosis of hypomagnesemia is based on history and signalment and secondarily confirmed with blood tests. Serum Mg concentrations below 1.5 mg/dl are indicative of potential problems, and values below 1 mg/dl are diagnostic. Magnesium concentrations can be measured in eye fluid (48 hours), urine (24 hours), or cerebrospinal fluid (12 hours) from samples collected for a limited time following death.[46]

Treatment

Slow intravenous administration (50–75 ml) of combined Mg and Ca solution is required for a positive response to therapy.[46] As with any Ca therapy, heart rate and rhythm should be monitored. Response to intravenous therapy is rapid, but may be short-lived. Repeat treatments may be necessary. Subcutaneous injection of Mg sulfate (25–50 ml, 50%) solution can help ensure blood Mg concentration over a longer period of time following intravenous therapy. Oral Mg supplementation should follow treatment to also help prevent relapse as well as ensure continuing adequate intake.

Prevention

Agronomic practices from modifying fertilization practices, applying dolomitic limestone, or seeding legumes

into grass pastures are all beneficial in preventing hypomagnesemia, but may not be economically practical. Appropriate dietary supplementation of Mg from late pregnancy through early lactation is needed. Dietary Mg should be increased to account for high dietary K, up to a point. Dietary Mg should not exceed 0.4% of dry matter. A ratio of dietary K to Mg of 4 : 1 is suggested. Magnesium can be supplemented in mineral mixes, but it is unpalatable. Molasses-based mineral blocks or licks should contain at least 5% Mg to meet requirements. Mixing 1 part magnesium oxide, 1 part trace mineral salt, and 1 part soybean meal or other palatable feed has been shown to be effective in maintaining good magnesium intakes and preventing disease problems.[46]

RUMINAL ACIDOSIS

Ingestion of large amounts of readily fermented sugars and starch, often in conjunction with reduced dietary effective fiber intake, results in excessive production of volatile fatty acids (VFAs) and lactic acid, overwhelming ruminal buffering systems, culminating in severely depressed rumen pH. Cereal grains are often implicated, but readily fermentable carbohydrates capable of inducing acidosis can be found in many byproduct feeds including bakery or candy waste, fruit pomace, beets, or potatoes.[41] Sugar and starch fermentability is related to the physical and chemical form of the compound and degree of processing. Corn starch, primarily amylase, is relatively less undegradable compared to wheat or barley starch, but if ground, ensiled, extruded, or flaked, rate of degradation increases markedly.

Clinical lactic acidosis may occur at any time, but is most common in early lactation in does not accustomed to high carbohydrate rations. Changing grain source or using processed grains may also predispose to acidosis if degradability properties of the concentrate have been increased. Many lactating does consume concentrate mixes free-choice during milking, and any event that increases the time spent in the milking barn may result in acidosis in some individuals. Subclinical acidosis is also likely to be common in dairy goats that are fed to maximize milk production.

Clinical forms of ruminal acidosis can present as peracute, acute, subacute, or chronic disease processes. Lactic acid is one of many potential fermentation end products generated by sugar and starch fermenting bacteria. Under normal rumen conditions, production of lactic acid is counterbalanced with its consumption by lactate fermenting bacteria. Lactic acid is a potentially deleterious product in the rumen, as it will reduce pH to a point of suppressing bacteria responsible for fiber fermentation. Most bacteria in the rumen are pH sensitive, not being able to survive below pH of 6.0. As the rumen pH declines, *Lactobacillus* bacteria will start to proliferate, generating d-lactic acid, which is more slowly metabolized by the host animal, creating a destructive downward pH spiral. The host animal succumbs to metabolic acidosis and hypovolemic shock as a result of fluid losses to hyperosmotic rumen contents.[41]

Clinical Signs and Diagnosis

Clinical signs are dependent upon acidosis severity and duration, which is a function of type and amount of feeds consumed, particle size and degradability of carbohydrates, and degree of adaptation to the diet. In subacute acidosis, clinical signs may be vague and include some or all of the following: feed refusal, especially grain; cyclic feed intake ("slug feeding"); reduction in milk fat composition; paste-like fecal pellets to mild diarrhea; and abnormal hoof growth (hardship rings). With peracute or acute acidosis, clinical signs may be recognized within hours to 1 day of consuming the inciting diet. Animals may present with anorexia, depression, ataxia, or weakness and appear "drunk." More severe peracute cases may present as recumbent with severe systemic signs of circulatory collapse and toxemia. Fluid accumulates in a static rumen, distending the abdomen and giving the rumen a "splashy" consistency on ballottement and induces various degrees of dehydration (sunken eyes, dry mouth, elevated packed cell volume, and total protein). Colic, teeth grinding, and diarrhea may be observed with some cases. Neurologic signs consistent with polioencephalomalacia—blindness, opisthotonos, head pressing, and seizures—may be present, depending upon the severity of dehydration, metabolic acidosis and toxemia present.[41] Bacterial endotoxemia results from rumen bacterial death and may lead to secondary hypocalcemia. Body temperature initially is elevated, but declines with disease progression. In severe cases, death occurs within 24 to 72 hours.

Diagnosis is confirmed by rumen fluid examination (pH and examination of flora) collected by ruminocentesis.[40,41] Subacute acidosis will have ruminal pH values below 5.5, whereas more severe cases may reach pH 4.0 or lower. Rumen contents will be milky gray in appearance and contain no or reduced numbers of protozoa and overwhelming numbers of gram-positive rods.[40,41] Urine and blood pH will also be low with high concentrations of lactate (>30 mg/dl). On necropsy, rumen contents will have a thin oatmeal consistency and particles of the offending feed consumed can be visualized. Rumen pH will decline continuously following death as a result of ongoing microbial fermentation; therefore, postmortem ruminal pH cannot be used to diagnose an antemortem condition.

Treatment

With mild to moderate cases, symptomatic therapy with rumen alkalizing agents (magnesium oxide [10–20 g] or hydroxide [50 g], sodium bicarbonate [20 g]),[40] oral fluids, and supportive care along with a reduction in grain feeding may suffice. Treatment for acute acidosis cases is more heroic, aimed at correcting acid-base imbalances, dehydration, and toxemia. On clinical presentation, a decision must be made as to whether or not emptying of rumen contents will impact clinical progression. Rumenotomy should be undertaken if significant amounts of the offending feed are still available for fermentation within the rumen. If rumen contents are liquid, siphoning off the fluid with an orogastric tube

may be sufficient, followed by rumen alkalizing agents. Rumen transfaunation should be used to re-establish microbial floral population. Intensive supportive therapy includes intravenous fluids with Na bicarbonate (5% or calculated to need), correction of electrolyte abnormalities, systemic antibiotics, and nonsteroidal anti-inflammatory agents. Sodium bicarbonate (1 g equals 12 mEq bicarbonate) required is calculated by multiplying measured base deficit times 0.3 and body weight (kg).[40] Systemic penicillin is the antimicrobial agent of choice.[41] Oral fluids are not indicated in more severe cases, as their absorption is limited and they further contribute to rumen distention. Other supportive therapy may include subcutaneous injections of thiamin, B–vitamins, and calcium gluconate.[40] Even if one recovers an affected goat, secondary problems related to bacterial and fungal infections of the liver and rumen wall need to be addressed.

Prevention

The rumen system is best maintained on a consistent dietary regimen optimizing the feeding of forage. Feed changes all need to be made gradually over several days so the flora have time to adapt, generally 10 to 21 days. Grain feeding within a diet must be controlled and managed appropriately. Dramatic changes in dietary forage-to-concentrate ratio, total amount of concentrate fed, and increased concentrate fermentability will be conducive to lactic acidosis. Feeding sequence should always offer forages or high roughage supplements before feeding grain concentrates, as forage or fiber consumption will stimulate rumination and increased saliva and buffer production. Diets containing minimal fiber (<19% acid detergent fiber), fiber that is easily sorted out, or fiber sources chopped too finely are at greater risk for acidosis. In situations in which large quantities of grain concentrates are required (high production, poor quality forages), grain feedings should be distributed into two or preferably more feedings of no more than 300 g per meal.[40] Inadvertent access to grain storage must be prevented.

Addition of rumen buffers, Na bicarbonate (1% of concentrate, 25 g/doe/day), or Mg oxide (0.4% of concentrate, 10 g/doe/day) can be added to the concentrate meal. These rumen buffers can have an additive effect when combined (2 to 3 parts Na bicarbonate to 1 part Mg oxide).[53] Dietary buffers can mitigate risk of acidosis and increased milk yield and milk fat content. Availability of free choice Na bicarbonate has been advocated for early lactation does. Although observations suggest adequate consumption of free-choice bicarbonate, does are more likely consuming the bicarbonate for the Na and may reduce their intake of free-choice trace mineral salt if it is being offered. Ionophore agents such as sodium monensin have been shown to reduce rumen lactic acid production and risk of acidosis by altering rumen microflora populations.[54] Although inclusion of sodium monensin (Rumensin, Elanco) has been recently approved for lactating dairy cow diets, it has not been approved for use in lactating goats' diets.

LOW MILK FAT SYNDROME

Milk composition is an important aspect of dairy goat production, as it influences income and quality of product. Low milk fat syndrome is a commonly encountered problem in dairy cows and one frequently observed in dairy goat production. Low milk fat is defined as a milk fat content well below standards for a breed with possible inversions of milk protein and fat content. For most dairy goat breeds milk fat percentage is typically between 3.8% and 4.2%. In low milk fat syndrome, milk fat content may decline below 3%.

The phenomenon of low milk fat syndrome has been well studied and a number of hypotheses have been proposed as to the cause.[55] Recent research with dairy cattle has unified two observations as to dietary causes of low milk fat syndrome. Diets low in fiber and high in grain can produce milk with low fat content. Similarly, diets with higher polyunsaturated fats can cause the same problem. Currently research suggests an intermediate compound resultant from rumen biohydrogenation of dietary polyunsaturated fatty acids produces a family of compounds identified as conjugated linoleic acids (CLA).[55] Many isomers of CLA have been identified, but one, trans-10, cis-12 CLA, has been shown to suppress fat synthesis in the mammary gland. The suppression in milk fat production is dose-dependent to the amount of trans-10 CLA. Recently reported research has shown identical mechanisms of altered CLA production with milk fat suppression when feeding high grain and polyunsaturated fat sources to lactating goats.[56,57]

There is no treatment other than to prevent the formation of trans-10 CLA in the rumen. Low-fiber diets with high grain intake seem to initiate the right rumen conditions to generate more trans-10 CLA. Feeding diets higher in vegetable fats will also increase trans-10 CLA production. Preventive practices should focus on appropriate fiber to concentrate levels in the diet and minimizing additional vegetable fat supplementation (whole soybeans, sunflower seeds, linseed oil, whole cottonseed).

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CHAPTER 76

Periparturient Infections and Structural Abnormalities

WILLIAM BRAUN, JR.

A variety of periparturient conditions may interfere with normal parturition or adversely affect the health or fertility of does after parturition. Fortunately, these conditions are not encountered frequently in goats but they are of grave concern to owners when they do occur.

HYDROPS

Excessive accumulation of fluid in the amniotic (hydramnios) or the allantoic (hydrallantois) sacs are infrequent complications of pregnancy. They are common in cattle but are rare in other species,[1] although there are two published reports of hydrops in goats.[2,3] The underlying cause of this condition is not known, but hydrops is encountered in the latter half of pregnancy and is characterized by rapid and progressive abdominal distention. The distention is a reflection of the accumulation of excess fluid within one or the other of the placental compartments. Normal fluid amounts in the pregnant goat uterus are 0.5 to 1.5 L, which may be increased greater than 10-fold in cases of hydrops. Other clinical signs are the result of compression of other organs by the expanding uterus and include a decrease in appetite, tachycardia, tachypnea, dyspnea, generalized weakness, and, finally, recumbency.

The excess fluid that accumulates in hydrops has an electrolyte composition more similar to extracellular fluid than to amniotic or allantoic fluids. In cows, an abnormal fetus (hydramnios) or placenta (hydrallantois) is associated with excessive accumulation of fluids. In goats, the fetus may have congenital abnormalities, be underdeveloped, or be apparently normal but not viable. The placenta has gross and microscopic pathologic changes, most commonly edema and necrosis. The placentomes are reduced in number and are structurally abnormal.

Diagnosis is based on presenting signs and the results of a physical examination. Differential diagnosis includes intestinal obstruction, ascites, ruminal tympany, hydrometra, and normal multiple fetuses.[2] Palpation and ballottement of the abdomen reveal the fluid accumulation, whereas ultrasonography shows that the accumulation of fluid is within a thin-walled uterus. Rectal palpation, as used for cattle, is impractical for diagnosis

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in goats. Ultrasonography can be used to demonstrate abnormal placentation in hydrallantois, abnormal placentomes, and a decrease in placentome numbers.

Treatment is directed at evacuation of the uterus by cesarean section or induced termination of pregnancy with prostaglandin. Uterine distention predisposes to uterine inertia, and assistance is necessary to remove the fetuses from the uterus. Cesarean section may be contraindicated in these cases as it risks uterine rupture, cardiovascular problems, and potentially fatal hypotension, all from the rapid loss of uterine fluids. Intravenous fluid therapy may be a useful adjunct therapy. Retained placenta and metritis are possible sequelae, requiring therapy after delivery.

VAGINAL PROLAPSE

Vaginal prolapse is fairly common, although not as common as in cattle or sheep. One author claims that it is seen most often in Saanens.[4] The predisposing causes are thought to be similar to those in cattle or sheep and include heredity, increased intra-abdominal pressure from advanced pregnancy, excess estrogen in the forage, previous dystocia, and relaxed perineal tissues due to confinement. If left uncorrected, vaginal prolapse will progress in severity and ultimately result in dystocia.

Early or mild cases are seen only when the doe is recumbent. A small, egg-sized portion of the vaginal floor protrudes through the vulvar lips and disappears into its normal position when the doe rises. Treatment often is not necessary in these cases, although the owner should watch these does to determine whether the condition progresses. Oily antibiotic preparations can be applied to keep the tissues moist and the doe can be confined at night with her hindquarters elevated. Adequate exercise can also be beneficial.

Complete vaginal prolapse does not correct itself spontaneously. The floor and walls of the vagina protrude from the vulvar lips and often result in tenesmus, which further aggravates the condition. The exposed tissue is prone to trauma and may become lacerated and infected. Enlargement due to edema occurs because of disruption of the vasculature of the vagina when prolapsed. In some cases, the urinary bladder is trapped within the prolapse and cannot be emptied, and thereby causes further enlargement of the prolapse. A vaginal-cervical prolapse occurs when the cervix becomes exteriorized along with the vagina. The cervical seal may be dislodged with vaginal-cervical prolapse, exposing the uterus to possible infection.

Treatment of vaginal prolapse entails cleaning the exposed tissue, decreasing its size, and replacing it into normal position. Epidural anesthesia assists replacement of the prolapse and helps to relieve tenesmus after replacement. The vagina is cleaned with mild soap and lubricated with an emollient. Elevation of the exposed tissue assists in evacuation of the bladder. Application of diffuse pressure to the organ, such as with a towel, and elevation of the hindquarters can reduce some of the edema. The vagina is replaced, making sure that fingertip pressure, a potential cause of lacerations, is not used.

After replacement, the vagina is retained in some manner. A paddle-shaped ewe prolapse retainer can be inserted to hold the vagina in place. This instrument does not interfere with parturition but may be difficult to hold in place in goats. Various suture patterns have been used to restrict the vulvar opening, thus preventing a subsequent prolapse. These need to be monitored carefully and opened prior to kidding, or dystocia will result. A shoelace pattern has been advocated because it has the advantage of being easily opened by the owner during parturition but can be left in place to secure the vagina after delivery. A modified Buhner suture secured by a bow knot accomplishes the same goal. Nonsteroidal analgesics, such as flunixin meglumine (1.1 mg/kg IM, daily), may suppress straining after replacement of the prolapse.

Prevention of vaginal prolapse is based on culling previously affected individuals. Once a doe suffers a vaginal prolapse, the prolapse will be repeated during each subsequent pregnancy. Some owners may not want to cull these goats, so they should be advised to watch affected does carefully during late pregnancy. Sexual rest for several years may allow the vaginal tissues to heal. Adequate exercise and prevention of obesity are also important to reduce recurrence.

UTERINE PROLAPSE

Uterine prolapse occurs infrequently after parturition in goats. The entire uterus is everted, often with the placenta still attached. Underlying causes include dystocia, uterine inertia, hypocalcemia, and a lack of exercise. Prepartum vaginal prolapse does not predispose to postpartum uterine prolapse. If the exposed tissue is not badly traumatized, the prognosis is usually good, even in cases of 24 hours' duration.[5]

The exposed tissue should be washed and as much of the placenta removed as possible, providing that trauma to the caruncles is avoided. Straining may inhibit replacement, so epidural anesthesia should be employed. To assist replacement, the exposed uterus and the hindquarters of the goat should be elevated. The uterus is then replaced using the same principles as replacement of a vaginal prolapse. The tips of the uterine horns should be completely returned to their normal position. This may be facilitated by uterine lavage, which allows fluid and gravity to assist in replacement of the tips of the uterine horns if they are not accessible to the operator's hand. Closure of the vulvar opening for several days may be warranted, as with vaginal prolapse.

Subsequent therapy includes oxytocin, systemic antibiotics for 3 to 5 days and tetanus prophylaxis. Lacerated and severely soiled prolapses may be complicated by metritis or peritonitis, so these cases should be closely monitored. Severe cases may require amputation of the uterus. Rupture of uterine blood vessels and hemorrhage are not common in goats. In uncomplicated cases, fertility is usually not reduced.

UTERINE RUPTURE

Spontaneous and induced rupture or tears in the uterine wall occur in goats. Most ruptures are associated with dys-

tocia and are cranial and ventral to the cervix. They occur as a result of manual traction placed on the fetus to effect delivery when fetal parts such as the head or shoulder exert pressure on the uterine wall and trap it against the maternal pelvis. Ruptures also occur in the dorsal wall of the body of the uterus from exuberant efforts to repel a fetus back into the uterus. On rare occasions the uterus may spontaneously rupture during late gestation and partially empty its contents into the abdomen, with subsequent peritonitis in some cases.

Tears or ruptures related to dystocia may be diagnosed when the uterus is explored for more fetuses. Little hemorrhage is evident in these cases. Minor tears can be treated by administration of oxytocin to hasten involution of the uterus. This is especially true for tears that occur in the dorsal wall of the uterus. More extensive ruptures may require manual eversion of the uterus and closure of the laceration with an appropriate suture. Systemic antibiotics and tetanus prophylaxis should be provided.

RETAINED PLACENTA

Fetal membranes are considered retained if they are not passed within 12 hours after the birth of the last kid. Diagnosis may be difficult in goats, as does often eat part or all of the placenta. Postpartum straining may indicate retained membranes or an undelivered fetus. Retained placenta is often associated with abortion or dystocia. Selenium deficiency has been incriminated in herd outbreaks of retained placenta.[6]

Diagnosis can be by the observation of the obvious tissue hanging from the vulva, or a vaginal speculum examination may be necessary. Retained placenta is treated by gentle attempts at manual removal or by hormone therapy. Vigorous attempts to manually extract the placenta should be avoided, as the caruncles may be damaged, causing excessive hemorrhage. Treatment with oxytocin (10–20 IU) at 2-hour intervals may be beneficial. Prostaglandin (5 mg $PGF_{2\alpha}$ IM or SC) may also assist in expulsion of the placenta and involution of the uterus. Metritis often accompanies retained placenta, and a regimen of systemic antibiotics for 3 to 5 days, or until the placenta is passed, is warranted. If the cervix is still open, intrauterine infusion of tetracycline (0.5–1.0 g IU) may be administered. Some clinicians advocate tetanus prophylaxis in cases of retained placenta.

METRITIS

Metritis is often a sequela to retained placenta or trauma to the uterus during dystocia. It may be an important cause of infertility in some goats. Acute, postpartum metritis is characterized by a malodorous, dark red uterine discharge. Does are febrile (40–41.5° C) and anorectic. Severe metritis may be complicated by peritonitis. Therapy includes systemic antibiotics and fluid therapy, if necessary. Repeated oxytocin or prostaglandin injections may facilitate uterine evacuation. Clostridial organisms, frequently *Clostridium tetani* and *Clostridium perfringens,* may colonize the uterus and cause fatal toxemia in affected goats. Tetanus and enterotoxemia prophylaxis, as adjunct therapy, may be prudent.

Chronic metritis or endometritis may occur after parturition or during the breeding season. Affected goats eat normally and are afebrile but have a whitish vaginal discharge. Chronic infections may be the result of postpartum metritis or the result of bacterial contamination during breeding. Systemic antibiotics and prostaglandin are the treatments of choice in these cases.

PYOMETRA

Pyometra is infrequent in goats. Anestrus, persistence of the corpus luteum, and an enlarged, exudate-filled uterus are the hallmarks of classic pyometra. Does give birth during the anovulatory season and do not resume estrous cycles again until many months later. Some Nubian and dwarf goats resume estrous cycles shortly after parturition and may develop pyometra. During the breeding season, cervical damage or bacterial infections introduced at breeding may progress to pyometra. Hydrometra or mucometra are far more common than pyometra in goats, and ultrasonography can be used to differentiate between these conditions. Pyometra is characterized by an echogenic, fluid-filled uterus as opposed to the echolucent intrauterine fluid characteristic of hydrometra. A purulent vaginal discharge may be present in some cases of pyometra. Treatment is the same as for chronic metritis.

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CHAPTER 77

Infectious Causes of Abortion

SEYEDMEHDI MOBINI

The female goat is normally a very fertile animal[1,2]; however, goats have a high incidence of abortion when compared with other farm animals. Infectious causes of abortion play an important role and could be a major source of economic loss in a goat herd. In an abortion outbreak, the safest approach is to assume that all causes of abortion are infectious in nature and to proceed from there.[3,4] Several microbial agents have been incriminated as causes of abortion in the goat, but the most common ones are *Chlamydia psittaci, Toxoplasma gondii, Campylobacter* spp., *Mycoplasma* spp., *Coxiella burnetii,* and *Brucella melitensis*. In the United States, chlamydiosis and toxoplasmosis are the most frequently identified infectious causes of caprine abortion.[5,6] In a study of 1784 abortion and stillbirth specimens submitted during a 10-year period, *T. gondii, Campylobacter* spp., and *C. psittaci* caused about 25% of all abortions in sheep.[7] Many of the organisms that cause abortion in sheep also cause abortions in goats, with the exception of campylobacteriosis, which is rare in goats in North America.[6]

The lesion common in all cases of infectious abortion is placentitis. Because of placentitis, the fetus either dies due to inability to exchange nutrients through the placenta, or becomes infected and dies.[4] Economically and emotionally, midterm or late abortions are of great concern to the owner, whether the animal is on a commercial or hobby farm, because the fetuses are lost and the unproductive female must be maintained until next breeding season or sold at a loss. A prolonged period of uterine disease and infertility may follow and in cases of infectious abortion, the disease threatens the rest of the herd.[8]

This section reviews the most common infectious causes of fetal loss in the goat.

CHLAMYDIOSIS

Chlamydiosis is the most common cause of infectious abortion in goats in the United States.[4,9] The disease is also one of the major causes of reproductive wastage in sheep breeding.[10] The disease has been described in Scotland, England, and in the western United States.[11] Chlamydia also causes pneumonia, keratoconjunctivitis, epididymitis, and polyarthritis in sheep and goats.[8,10,12,13]

Etiology

C. psittaci, a gram-negative, intracellular organism that contains both RNA and DNA, is the agent responsible for chlamydiosis.[5] This organism was established as the causative agent of enzootic abortion of ewes in the late 1950s.[14]

The antigenic strains found in goats appear to be closely related to those in sheep. Antigenic type 1 is implicated in abortions, birth of stillborn or weak kids, and in neonatal chlamydial pneumonia, whereas type 2 isolates cause polyarthritis and conjunctivitis in adult goats.[15–17] Serotype 2 has been recently reported as an abortion-inducing strain in ruminants as well.[10] There is no cross protection between these two antigenic types.[15]

Epidemiology and Pathogenesis

Pigeons and sparrows can serve as reservoirs for the organism, and it has been suggested that ticks or insects may play a role in transmission of the disease.[16,17] *C. psittaci* can persist in the feces of infected animals, which may explain why the infection carries over from one kidding season to the next.[10,14] Aborting does shed large numbers of organisms from the uterine discharge, fetus, and placenta, particularly during the first 3 weeks after abortion. Elimination of chlamydiae from the uterus is complete within 3 months.[18,19] Inflammation and necrosis caused by multiplication of the chlamydiae prevent normal transfer of nutrients across the placenta, and the fetus dies and is aborted.[5] Does exposed to *C. psittaci* for the first time in the first half of gestation abort that pregnancy, whereas susceptible does exposed for the first time during the last half of pregnancy usually abort during the subsequent pregnancy.[20] Regardless of the time of infection in the female, the organism does not begin to proliferate and attack the placenta until about day 90 of gestation.[21] Even though chlamydiae have been isolated from the semen of experimentally infected rams for up to 29 days after inoculation, venereal transmission during breeding has not been investigated.[16,17]

There is no evidence of infertility during the subsequent breeding season in aborting does, and immunity after an abortion lasts about 3 years. Older does may abort because of chlamydiae repeatedly.[4,9] Outbreaks of abortion among goats by co-infection with *Coxiella burnetii* have also been reported.[22]

Clinical Signs

In the United States, 25% to 60% of does in endemically infected herds that are kidding for the first time abort.[9] Abortions usually occur during the last month of gestation but can occur as early as day 100 of gestation.[4,8,18,19] Does are not normally ill but may show a bloody vaginal discharge 2 to 3 days prior to abortion. The fetus may be

autolyzed or fresh. Some weak newborns are seen, and a few does may retain the placenta.[4,9] Pathologic changes in the fetus are nonspecific. The fetus may be delivered in a fresh state but may be autolyzed if retained in the uterus for a day or two. The placenta shows regional to generalized placentitis (white to yellow necrotic areas) involving the cotyledons and intercotyledonary space.[4,9]

Diagnosis

Diagnosis is based on a history of abortion along with clinical signs and demonstration of characteristic inclusion bodies in impression smears of placenta, fetal tissue, or uterine discharge. A definitive diagnosis is made by culturing the organism from the placenta or fetal tissue.[4,9,12,13] Serologic testing is also a valuable aid in diagnosis. Ewes, does, and cows have significant rises in antibodies against chlamydial antigen after abortion.[23] Paired blood samples from the doe, 2 to 3 weeks apart, are necessary for serologic tests.[4,13,14] Antibodies may be detected in fetal serum as well. The tests of choice are the enzyme-linked immunosorbent assay and the indirect inclusion fluorescent antibody test.[23] Titers in the range of 1:32 to 1:256 suggest chlamydial infection. A fourfold increase in antibody titer between paired serum samples is significant.[14]

When chlamydiosis is suspected, the following samples should be submitted for diagnostic evaluation[14]:

1. Fresh placenta and fetus packed in ice.
2. Vaginal swab taken within 3 days of parturition, if the placenta is not available.
3. Paired serum samples from the aborting dam and a normal doe.

Treatment and Control

Limited success has been achieved in controlling an outbreak by treatment of all females with tetracycline in the last 4 to 6 weeks of gestation.[4,9,13] Suppression of the organism may prevent additional placental damage and also reduce shedding of chlamydiae by treated does. Large herds are commonly treated with oral tetracycline (400 to 500 mg per head per day) for 2 weeks.[5,24] This would be a reasonable approach for fiber-producing does. In dairy herds, it is more customary to treat individual nonlactating does by injection of long-acting oxytetracycline at a dose of 20 mg/kg IM every 10 to 14 days.[5] Other authors have given the drug twice a week in the last 4 to 6 weeks of gestation.[4,9,13] Considering the management difficulties and cost associated with prevention, the most effective protocol appears to be one injection of long-acting oxytetracycline at 6 to 8 weeks prior to parturition, followed by a second injection 3 weeks after parturition.[17]

Enzootic abortion is of such serious economic consequence in some countries that compulsory government vaccination programs have been implemented.[11] A killed vaccine for sheep is available in the United States and can be used in goats (extra-label use) but may cause a local as well as a systemic reaction for several days (marked soreness and stiffness).[6,25] The vaccine is usually available only in combination with *Campylobacter* bacterin or *Campylobacter* and *Escherichia coli* bacterin (*Campylobacter fetus–Chlamydia psittaci–Escherichia coli* bacterin*).[6,25] The vaccine should be given IM or SC 8 weeks prior to breeding and followed in 4 weeks with a second vaccination.[25] Even though trials in sheep have shown that protection from abortion lasts for about 3 years, annual revaccination is recommended.[25,26] Vaccination helps to prevent abortion, but it does not eliminate infection.[5] Aborting does should be removed from the herd for at least 3 weeks, and the fetus and placenta should be burned or buried. Care must be taken to prevent contamination of feed and water. No feed should be given on the ground, and feeders should be designed to prevent goats from getting into them.[11]

Zoonotic Potential

Chlamydia psittaci is contagious to humans. During kidding season, pregnant women assisting with parturition may become infected and abort. An influenza-like syndrome has also occurred in men assisting with lambing in infected flocks.[5] Veterinarians and farmers attending normal parturition, dystocias, or abortions should wear plastic gloves to limit exposure to uterine fluids. The same precautions apply when collecting fetuses or placentas for disposal or diagnostic evaluation. Pregnant women should avoid contact with the herd during the kidding season.[5]

TOXOPLASMOSIS

Toxoplasmosis is one of the most common parasitic infections in goats, other livestock, and humans worldwide.[27,28] Toxoplasmosis is a major cause of abortion in sheep in New Zealand and England and is known to cause reproductive losses in goats and other animals.[29]

Etiology

The protozoan *Toxoplasma gondii* is the agent that causes abortion, mummification, stillbirth, and birth of weak kids in goats as well as in sheep.[5,27]

Epidemiology and Pathogenesis

Cats are pivotal in the transmission of *T. gondii*.[30] They become infected by ingestion of infected rodents and birds, which can lead to excretion of large numbers of environmentally resistant oocytes. Transplacental infection can develop in cats, and kittens infected in utero can shed *T. gondii* oocytes after birth.[30] Cats often defecate and bury their feces in the hay and food bins of barns. Does become infected by ingestion of food or water contaminated with oocytes from feces of infected cats. The organism enters the blood and spreads to other tissues within 2 weeks of ingestion of oocytes. In pregnant does, toxoplasmas can invade and multiply in the placenta and then spread to the fetus, causing abortion, fetal death,

*Pfizer, Animal Health, Exton, PA.

resorption of the fetus, stillbirth, birth of a live but weak kid, or birth of a normal kid, depending on the stage of pregnancy.[27] Nothing is known about the breed susceptibility to toxoplasmosis in goats. Although *T. gondii* can be found in goat semen, it is doubtful that venereal transmission is important in causing abortion.[28]

Clinical Signs

Goats appear to be more susceptible to toxoplasma infection than sheep.[31] Abortion can occur in does of all ages but primarily in does that acquire infection during pregnancy. Abortion may be repeated in the next gestation.[28] The incidence of abortion in a flock may vary from 3% to 30%. Ewes and does infected prior to breeding do not abort. Those infected 30 to 90 days after breeding usually have fetal resorption or mummification. Most observed abortions occur in the last trimester of gestation, 2 to 3 weeks before term, after infection during the latter half of gestation.[8] The does themselves are generally clinically normal at the time of abortion.[28]

Abortion occurs because of necrosis of the placenta, particularly the cotyledons, or fever in the doe. The intercotyledonary areas of the placenta are usually normal, with the cotyledons having white to yellow focal areas of necrosis and calcification up to 1 cm in diameter (Fig. 77-1). These lesions are clearly visible when the cotyledons are washed in saline. The lesion is characteristic of only toxoplasmosis and can be used in the field as a diagnostic tool.[4,8,13,28,29] On the other hand, *C. burnetii, Brucella* spp., and *Chlamydia* spp. usually cause placentitis that includes the intercotyledonary region.[29]

Diagnosis

Rapid diagnosis of infectious abortion in goats is important. Several tests are available for diagnosis. A presumptive diagnosis can be made from placental lesions alone; however, placenta often is not available for examination because it may be decomposed.[31]

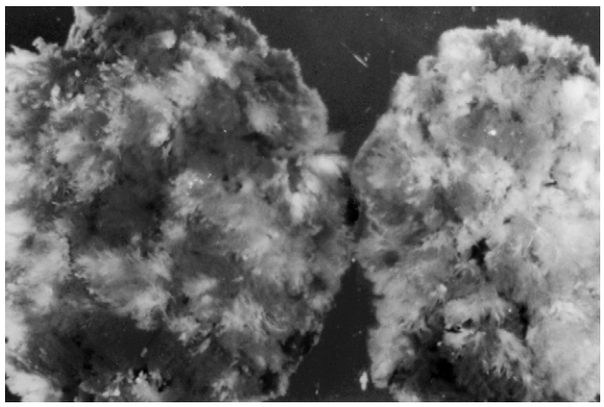

Fig. 77-1 White to yellow focal areas of necrosis in fetal cotyledons of does naturally infected with *Toxoplasma gondii.* (From Dubey JP: Toxoplasmosis: zoonosis update. *J Am Vet Med Assoc* 1990;196:123.)

The preferred diagnostic procedure is determination of *T. gondii* antibody in fetal fluids or presuckling serum. Their presence indicates transplacental toxoplasma infection.[32] The absence of *T. gondii* antibody does not preclude the possibility of infection, however. It is important to ensure that serologic examination of kids is done before colostrum is given because the amount of antibody in colostrum is several times greater than that in serum and may cause high neonatal antibody titers.[28] The presence of high antibody titers in doe serum is not necessarily diagnostic of recent infection because titers may remain high into the next breeding season. However, absence of antibodies is considered to be conclusive evidence that toxoplasmosis was not the cause of abortion.[5,28] The modified agglutination test can be used to detect antibody in fetal and maternal serum. The modified agglutination test is easy to perform and more sensitive than other tests.[32] The enzyme-linked immunosorbent assay and the indirect fluorescent antibody tests have also been used.[28]

Positive diagnosis of toxoplasmosis requires isolation of the organism from the placenta or fetal brain, lung, and muscles.[32] The samples for isolation studies should be shipped on ice but not frozen.[28]

Treatment and Control

Control of toxoplasmosis is based on preventing exposure of goats to cat feces. Cats should be prevented from defecating in feeders or in hay. Fetal membranes and dead fetuses should be buried or incinerated to prevent infection of cats and other animals.[30] Cats should not be allowed near pregnant sheep and goats, but in practice this is difficult.[28,30] A vaccine that contains a strain of tachyzoites (S48) that do not persist in tissues of sheep is available in Europe and New Zealand, but it is not available in the United States. Ewes vaccinated with the S48-strain vaccine remain immune for at least 18 months.[30] Breeding goats that have been exposed to *T. gondii* infection are likely to be relatively resistant to the effects of reexposure during subsequent pregnancies and are less likely to abort again from this cause and therefore should not be culled.[33]

Zoonotic Potential

Fetal membranes and dead fetuses from sheep or goats that abort should be handled with caution by persons wearing gloves to prevent infection in humans.[30] Toxoplasmosis has been reported in human beings after drinking raw goat milk. Therefore, goat milk should be pasteurized or boiled before human consumption. This is particularly true for its use in infants, who are more susceptible to toxoplasmosis than adults.[34]

Q FEVER

Query or Queensland fever is a zoonotic infection affecting a variety of animal species and human beings. It can be the cause of abortion in sheep and goats[5,35] and has been reported in Canada, the United States, and many other countries.[4,5,8,35,36]

Etiology

Q fever is caused by *C. burnetii,* an obligate intracellular rickettsial organism.

Epidemiology and Pathogenesis

Cattle, sheep, goats, and wildlife can carry the organism, which is shed in large numbers in placentas, uterine fluids, colostrum, and milk.[5,36] Therefore, cows and sheep may be a source of infection for goats when they share a pasture. Animals and humans can be infected by inhaling contaminated dust. Contaminated grazing pastures and tick bites are other modes of transmission.[5]

Clinical Signs

The primary significance of this disease is its zoonotic potential. In livestock, the disease is usually unapparent; however, occasional abortion outbreaks due to *C. burnetii* have been reported in sheep and goats.[6,37] Clinical signs are infrequent, but abortion or stillbirth usually occurs late in gestation owing to severe damage to the placenta and necrosis of the cotyledons and thickening of the intercotyledonary areas.[4,36,37] Some does abort without apparent clinical signs, while others show anorexia and depression 1 to 2 days before abortion.

Diagnosis

Diagnosis is based on placental findings, serology, and isolation of the organism.[4,36] Isolation of *C. burnetii* is the ideal means for diagnosis of the disease, but it is usually not feasible because of the contagious and zoonotic potential of the organism. Very few diagnostic laboratories are willing to handle this organism because of strict biosafety procedures.[37] The fluorescent antibody test can be used to identify the organism in frozen sections of placenta. Even though a variety of serologic tests have been described, asymptomatic infections without abortion are possible and a diagnosis of abortion cannot be given solely on the basis of positive serologic test results.[38] Antibody titers of 1:20 or higher indicate exposure to *C. burnetii*. A fourfold increase in antibody titer between acute and convalescent samples indicates recent infection.[37] A rapid presumptive diagnosis of *C. burnetii* is possible by identification of large number of characteristic organisms in the placental tissue together with elimination of other causes of placentitis such as *Brucella, Campylobacter,* and *Chlamydia* spp.[6,37]

Treatment and Control

Once an infection is established, the organism is carried by the doe indefinitely and is shed in the milk and at parturition. Placenta and aborted fetuses should be burned or buried.[36] There is no vaccine for goats. In cattle, treatment with tetracycline for a period of 2 weeks has been shown to prevent excretion of the organism at parturition. In an outbreak of Q fever abortion in goats, abortions stopped following feeding of 200 mg per animal per day of chlortetracycline in the feed for 19 days.[36] Some authors recommend injection of 20 mg/kg of long-acting oxytetracycline every 3 days or every 10 to 14 days.[6]

Zoonotic Potential

Q fever is a zoonotic disease transmitted to humans by milk, placenta, and feces. The disease is characterized in humans by influenza-like symptoms.[12,36] In the majority of human cases, there is a history of contact with infected sheep, goats, or cattle.[37] The organism is killed by pasteurization but is readily transmitted by nonpasteurized milk. A mask should be worn when manure is removed from barns and gloves should be worn at time of kidding or when handling an aborted fetus.[5]

LISTERIOSIS

Listeriosis most commonly causes meningoencephalitis but can also cause abortion and septicemia in goats.

Etiology

Listeria monocytogenes, a gram-positive, non–acid-fast organism, is the agent responsible for listeriosis. Abortion-causing strains are often serotype 1.[5]

Epidemiology and Pathogenesis

Listeria monocytogenes may be found in soil, water, plant litter, silage, and the digestive tract of ruminants and humans.[5] The organism can survive in soil and feces for a very long time and grows in poorly fermented silage (pH > 5.5).[2,4,5,8] On some farms abortion is attributed to silage feeding, but abortions have been reported on farms on which the goats were fed hay with the addition of concentrate.[5,39]

Clinical Signs

Abortion results from infection early in gestation, but later infection results in stillbirth or weak kids. The abortion form and the encephalitic form of listeriosis do not usually occur simultaneously in a herd.[5] Abortion occurs during the last 2 months of pregnancy, preceded by septicemia.[4,5] Signs of septicemia may include fever, decreased appetite, and reduced milk production. Kids grafted to aborting does may die of listerial septicemia contracted through the milk.[5]

Diagnosis

Diagnosis is based on culture of the organism from the placenta, aborted fetal tissue, and vaginal discharge. High serum antibody titer to *L. monocytogenes* in goats that have aborted is indicative of listeriosis as a probable causative agent of abortion.[39]

Treatment and Control

Feeding of poor-quality or spoiled silage should be discontinued. Vaccination with the aim of producing cellular immunity has been investigated. Theoretically, a live vaccine should be more effective than killed preparation.

Two doses of reduced-virulence live vaccine before breeding is reported to have provided significant protection against experimental challenge in pregnant does.[5]

LEPTOSPIROSIS

Sheep and goats are among the domestic species that are less susceptible to leptospirosis. There are very few investigations of the incidence of leptospirosis in these species.

Etiology and Epidemiology

Several serotypes of *Leptospira interrogans* have been shown to cause abortion in goats, but the prevalence of losses is not known.[5] Other *Leptospira* serotypes such as *L. icterohaemorrhagiae, L. grippotyphosa,* and *L. pomona* have been reported to be the main causes of leptospirosis in goats.[40] Goats appear to be more susceptible to leptospirosis than sheep.[40] Goats probably do not serve as primary reservoirs for leptospirosis, and infection probably occurs from exposure to an environment contaminated by the urine of other species.[5] In a study of 262 herds of goats with abortion in Spain, 2.6% revealed leptospires as the causative agent, with serotype *L. pomona* being the most prevalent (75%), followed by *L. sejroe* (12.5%), and *L. icterohaemorrhagiae* (12.5%).[40]

Clinical Signs

Clinical signs include anorexia, fever, marked jaundice, hemoglobinuria, anemia, nervous signs, and abortion.[5,40] Abortions have been reported during the last trimester of gestation.[40]

Diagnosis and Control

Dark-field microscopy, immunofluorescence testing, and silver stains of placenta and fetal tissue and fluids are used to confirm the diagnosis.[5,40] The organism is difficult to isolate from contaminated specimens. Paired sera from does that have aborted showing an increase in titer would be suggestive of abortion associated with leptospirosis.[5]

Vaccination twice a year is indicated in regions where leptospirosis is prevalent in goats. Other measures recommended include separation of animal species, controlling rodents, and maintaining a clean water supply.[5]

OTHER INFECTIOUS CAUSES OF ABORTION

Any systemic illness that induces a febrile response or generalized illness such as septicemia and toxemia may cause abortion in does. Some of the disease conditions that lead to occasional abortions are briefly described in the following sections.

Mycoplasmosis

Mycoplasma spp. are important pathogens of goats and cause mastitis, arthritis, keratoconjunctivitis, and occasionally vulvovaginitis and abortion.[8,41] Abortion is not the dominant clinical finding in an outbreak. *Mycoplasma mycoides* and *M. agalactia* have been reported to cause abortion in goats in the United States.

Abortion occurs in does during the last trimester of gestation. Does that abort will probably have the organism present in their milk, liver, kidney, spleen, amniotic fluids, and placenta. The organism may also be found in cotyledons, liver, and spleen of the fetus. Diagnosis of abortion due to *Mycoplasma* spp. is by culture and serotyping of the isolate.[41]

In other countries, treatment with tetracycline or tylosin may be preferred for economic reasons, but in the United States slaughter is recommended.[41]

Campylobacteriosis

Campylobacter (formerly *Vibrio*) is the most significant cause of abortion in sheep in the United States.[8,13] However, *Campylobacter* abortion has been rarely documented as the cause of abortion in goats in North America.[5,8,42]

Etiology

Of the cases reported in the United States, *Campylobacter jejuni* and *C. fetus* (formerly *Vibrio fetus intestinalis*) have been identified as the causative organisms.[5,42]

Clinical Signs

Clinical signs include late-gestation abortions in does and weak or stillborn kids. Aborting does may or may not show signs of systemic illness. A mucopurulent or sanguinopurulent vaginal discharge is reported in all aborting does.[42] In South Africa, where *Campylobacter* abortion appears to be common, as many as 30% of aborted kids have grossly visible liver necrosis. The placenta is often edematous, with necrosis of cotyledons.[5]

Diagnosis

Definitive diagnosis of *Campylobacter* abortion is by isolation of the organism. Direct microscopic examination and isolation of *Campylobacter* spp. from placenta, fetal abomasal contents, and maternal vaginal discharge is the preferred diagnostic procedure.[43] Serologic tests can be done at a few specialized laboratories.

Treatment and Control

Treatment is similar to that used in sheep. The antibiotic regimen is penicillin/streptomycin preparation or tetracycline in feed.[13,42] In a confirmed outbreak, vaccination of all pregnant does with an ovine *Campylobacter* bacterin is advisable.[5]

A combined killed bacterin of *C. fetus* and *C. jejuni* is available for use in sheep but not routinely used in goats. The vaccine is administered prior to breeding with a booster in 2 to 3 months. Revaccination annually shortly before or just after breeding is recommended.[25]

Because of the probable oral route of infection, sanitary conditions, avoiding fecal contamination of feed, and isolation of aborting does are recommended.[42] As with other abortions, placentas and aborted fetuses should be burned or buried deeply.

Zoonotic Potential

Campylobacter jejuni has been recognized as a cause of mild gastroenteritis in humans and is of public health importance. Domestic animals and unpasteurized milk are thought to be the source of *C. jejuni* infection in humans. Aborted fetuses infected with *C. jejuni* should be handled carefully. In Scotland, shepherds giving artificial resuscitation to infected lambs have acquired the disease.[42]

Brucellosis

Brucellosis is an infectious disease of goats characterized by abortion, the birth of weak kids, mastitis, and formation of localized lesions in various tissue.[44] The incidence of brucellosis in goats is extremely low to nonexistent in the United States, but the disease is widespread in the Middle East, India, Pakistan, Africa, Mexico, and parts of South America.[2,4,12] Sporadic outbreaks have been reported from Texas and Colorado.[2]

Etiology

Brucella melitensis, a small gram-negative coccobacillus specific for goats, is the cause of brucellosis in goats as well as cause of Malta fever in humans.[2,4,12] Occasionally, *Brucella abortus* infection occurs in goats running with infected cattle or after vaccination with strain 19 by ill-informed veterinarians, but it does not spread within the herd and is not readily transmitted between herds.[44]

Epidemiology and Pathogenesis

The most common route of transmission in goats is by ingestion of contaminated feed and water. The organism enters through mucous membranes and becomes localized in the lymph nodes, udder, uterus, testes, and spleen.[2,44] In pregnant animals, localization in the placenta leads to the development of placentitis with subsequent abortion. The organism is excreted in milk, urine, feces, and placenta and for 2 to 3 months in vaginal discharge. Kids born alive to infected does often are infected and capable of shedding the organism.[2,5,44]

Clinical Signs

Infection occurs in both sheep and goats, producing abortion during late pregnancy.[44,45] As in other species, there may be an "abortion storm" when the disease is introduced followed by a period of resistance during which abortions do not occur. A systemic reaction characterized by fever, depression, loss of weight, and sometimes diarrhea occurs and may be accompanied by mastitis, lameness, hygroma, and orchitis in males.[2,44] On gross examination, the placenta is normal.[2,3]

Diagnosis

A diagnosis of brucellosis as the cause of abortion is usually made by isolation of the organism from an aborted fetus, placenta, or vaginal discharge. Various agglutination, precipitation, and complement fixation tests are used to detect carrier goats.[5]

Treatment and Control

There is no treatment for brucellosis in goats. In countries where the prevalence of infection is very low, slaughter of the entire herd (both sheep and goats) would probably be the control measure of choice. This was the method successfully used to eradicate the outbreak reported in Texas.[2,5,45] In other situations, a test and slaughter program may be more appropriate. All new animals imported to a farm should be blood-tested, and bucks should be tested before they are used for breeding.[2] Placentas and aborted fetuses should be burned or buried deeply.

Vaccination of goats is not permitted in the United States. In most countries where caprine brucellosis is prevalent, the disease is controlled by an intensive vaccination program that is very effective. A live attenuated strain of *B. melitensis* Rev 1 is usually given subcutaneously to kids and lambs 3 to 8 months of age.[5,45] The vaccine will cause abortion, and thus is to be avoided in pregnant animals or those within 1 month of mating. Immunity from a single dose is considered to be lifelong.[5]

Zoonotic Potential

Goats were the first species associated with brucellosis in humans on the island of Malta.[44] Often, the first indication of brucellosis in goats is undulant fever in humans who consume unpasteurized milk or cheese.[2,5] Therefore, a large percentage of the infections occurring in humans can be prevented by pasteurization of goat dairy products.

Salmonellosis

Salmonella infection causes abortion, metritis, and systemic illness in does.[24] *S. abortus-ovis* was first implicated as a cause of abortion in 3- to 4-month pregnant goats in Cyprus. The organism also has been isolated from goat fetuses and placentas in France.[5] Only sheep and goats are affected by *S. abortus-ovis; S. typhimurium* and *S. dublin* both have been associated with abortion.[5]

Specific agglutinins can be demonstrated in the sera of adults in the herd and in aborted fetuses. Control measures include injecting pregnant does with tetracycline and administering two doses of vaccine, followed by an annual booster.[5]

Akabane Virus Disease

Akabane virus causes arthrogryposis and hydrencephaly in cattle. It has also been reported in goats.[2,8,12] The disease may cause abortion, stillbirth, and mummified fetuses in goats. It has been reported from Australia, Japan, South Africa, the Middle East, and Argentina.[2,8,12] The disease is exotic to the United States.[5]

Akabane virus is an arbovirus, transmitted by gnats and mosquitos.[5] Clinical disease has been observed in sheep, goats, and cattle. Infection of nonpregnant goats is subclinical. Pregnant goats may remain healthy, but abort or deliver stillborn kids. Veterinarians may first encounter Akabane disease when assisting a goat in labor, because dystocias are common when arthrogryposis is present (Fig. 77-2).[5]

Akabane virus disease is diagnosed on the basis of arthrogryposis and/or hydrencephaly in newborn kids and the identification of a positive antibody titer in those

Fig. 77-2 A fetus showing arthrogryposis and hydrencephaly delivered by cesarean section.

kids or aborted fetuses. There is no treatment for Akabane virus infection; however, effective vaccines have been developed and are used in advance of the breeding season in epizootic areas.[5]

Yersiniosis

Yersinia pseudotuberculosis is a zoonotic bacterium commonly carried by wild birds or rodents. Fecal-oral infection of goats can result in an enteric infection with subsequent bacteremia. Abortion and early neonatal death of kids have been reported in the United States. *Yersinia* has been recovered from placenta and fetal abomasal contents.[5,24,46]

Tick-borne Fever

Ehrlichia (Rickettsia) phagocytophilia, a tick-borne disease of sheep, goats, and cattle in the United Kingdom, Europe, Africa, and India causes fever, listlessness, decreased milk production, lameness, and abortion. Diagnosis is by demonstration of cytoplasmic inclusions in granulocytes and monocytes in the blood of does and by complement fixation tests. The organism does not invade the fetus, so smears of fetal tissues are not helpful.[5]

Sarcocystosis

Sarcocystis is a cyst-forming protozoan, frequently noted as an incidental finding on histologic examination of cardiac and skeletal muscle of many species. Experimental inoculation of goats, 75 to 105 days pregnant, with *Sarcocystis capracanis* resulted in illness and abortions.[5] Naturally occurring abortion and birth of stillborn kids by sarcocystosis has been reported in a herd of milking Saanen does in Australia.[47] Some of the does exhibited a vulvar discharge but no other clinical signs. The mechanism of *Sarcocystis*-induced abortion is unknown but may relate to maternal fever.

Anaplasmosis

Anaplasma ovis, a tick-borne disease of goats, usually causes a subclinical, mild, febrile disease. However, abortion has been reported in *Anaplasma*-infected Boer goats in a semiarid region of South Africa that had to walk long distance for food.[5]

SPORADIC CAUSES OF ABORTION

Other sporadic causes of abortion are reported in the literature and include a *Neospora*-like protozoal infection associated with abortion in two pygmy goats,[48] *Enterobacter cloacae* abortion in a flock of 35 goats,[49] *Actinomyces pyogenes,*[50] enterotoxemia caused by *Clostridium perfringens* type D,[24] foot and mouth disease, Nairobi sheep disease, Rift Valley fever, and Wesselsbron disease.[5]

DIAGNOSTIC INVESTIGATION OF CAPRINE ABORTIONS

A systematic approach is essential for the diagnosis of abortions in goats. Whenever losses continue or abortion storms occur, there is a need for a thorough investigation of the problem. Many different causes need to be considered and each requires special tests. The laboratory needs to be aware of the infectious agent most likely to be present in the area. The practitioner must obtain a good nutritional and clinical history, including possible exposure to carrier animals. Although history seldom provides information pointing directly to the cause of abortion, clues may be found that will indicate what needs to be done to determine the diagnosis. It is up to the clinician and the owner to provide a complete and accurate history. The owner should keep detailed records and accurately identify each aborting animal and the stage of gestation at which the animal aborted.

The first abortion in a herd may be the most important one, but many owners do not become seriously concerned until an abortion storm is evident. The owner should be instructed to freeze any fetus and placenta, no matter how certain he or she might be that the abortion was caused by fighting or a fall. When abortion occurs, the does should be isolated and any aborted fetus kept for further laboratory examination. Each diagnostic laboratory has slightly different preferences for specimens to be submitted. Therefore, the diagnostic laboratory should be consulted prior to sample submission.[51] In sheep and goats, the entire fetus, placenta, and paired serum samples from aborting does should be submitted to the diagnostic laboratory.[51] Without the placenta, identification of chlamydiosis and toxoplasmosis is unlikely. If the placenta and fetus cannot be quickly and suitably transported in their entirely, samples may be removed and one portion placed in a fixative and another in plastic containers packed on ice for culture. Tissues may be frozen for future viral culture or tested for agents using the fluorescent antibody technique. Samples from the fetus for culture should include caruncle, placenta, abomasal content, lung, liver, spleen, and kidney. Whenever possible, a fetus and placenta from several aborting does should be submitted. Mummified fetuses (a brownish,

Table **77-1**

Diagnostic Summary of Infectious Abortion in Goats

	EPIDEMIOLOGY			LABORATORY FINDINGS		
Disease	***Transmission***	***Time of Abortion***	***Clinical Data***	***Fetus***	***Serology***	***Vaccination***
Chlamydiosis *(C. psittaci)* type1	Ingestion	Last 2–3 weeks	Vaginal discharge before; retained placenta and metritis after; thickening and necrosis of cotyledonary and intercotyledonary tissue	Autolyzed or fresh	ELISA or IIFA	Killed vaccine 8 weeks prior to breeding followed by second dose 4 weeks later
Toxoplasmosis *(T. gondii)*	Ingestion	Last 2–3 weeks	Abortion, fetal death, stillbirth, neonatal death; multiple white to yellow focal areas of necrosis and calcification in fetal cotyledons; intercotyledonary area normal	Fresh or mummified	MAT	Strain S48 available in Europe or New Zealand
Q fever *(Coxiella burnetii)*	Ingestion, inhalation	Late in gestation	Abortion or stillbirth, cotyledonary and intercotyledonary necrosis and mineralization	—	FA of frozen placenta	None
Campylobacteriosis (*C. jejuni* and *C. fetus*)	Ingestion	Late in gestation	Metritis after abortion; placental edema, necrosis of cotyledons	Organism in fetal stomach content	In few specialized laboratories.	Combined killed bacterin prior to breeding
Brucellosis *(B. melitensis)*	Ingestion	Late in gestation	Fever, depression mastitis, orchitis, lameness, or abortion; placenta normal; not in US	Organism in placenta or fetal stomach	Complement fixation, agglutination	Live attenuated *B. melitensis* Rev 1 vaccine in prevalent areas; none in US
Listeriosis *(L. monocytogenes)* serotype 1	Ingestion, poor silage	Last 2 months	Septicemia, metritis, no placental lesion	Stillborn or weak	Agglutination	Live vaccine before breeding, if needed
Leptospirosis *(L. interrogans, L. pomona)*	Ingestion	Last trimester	Anorexia, fever, jaundice, anemia, abortion	Organism in fetal tissue	Immunofluorescence test	Vaccination in prevalent area
Mycoplasma (M. mycoides, M. agalactia)	Ingestion	Last trimester	Vulvovaginitis and abortion, mastitis, arthritis, keratoconjunctivitis	Organism in fetal cotyledons, liver, spleen	—	None
Salmonellosis *(S. abortus-ovis)*	Probably ingestion	Last 6 weeks	Abortion, metritis, systemic illness	Organism in fetus and placenta	Agglutination test	None
Akabane disease (virus)	Gnats and mosquitoes	Late abortion	Abortion, arthrogryposis and hydrencephaly in newborn kids; exotic to US	Stillborn or mummified	Antibody titer	Vaccine in epizootic area before breeding season

ELISA, enzyme-linked immunosorbent assay; FA, fluorescent antibody; IIFA, indirect inclusion fluorescence antibody; MAT, modified agglutination test.

Box **77-1**

Materials to Be Submitted to Diagnostic Laboratory for Abortion Investigation

Frozen or Refrigerated Tissues
- Whole fetus and entire placenta preferred
- Fetal lung, liver, kidney, spleen
- Fetal stomach content
- Dam's serum

Formalin-Fixed Tissues
- Fetal lung, liver, kidney, spleen
- Fetal brain
- Fetal heart and skeletal muscle
- Placenta

Other Data
- History
- Feed if indicated
- Water if indicated

hairless fetus with an elongated muzzle and sunken eyes) are unsuitable for either culture or histologic examination. Autolysis commonly obscures diagnostic changes. A summary of diagnostic materials that may be needed for an abortion investigation is presented in Box 77-1.

Paired serum samples may be required to confirm or deny suspicion of toxoplasmosis or chlamydiosis. Results of examination of a single serum sample from an animal that has aborted must be interpreted with great care, or confusion may result. Although certain titers are set as significant for disease regulation purposes, there is no specific titer for any infection that absolutely will differentiate infected from uninfected animals. Serologic results should always be correlated with herd history, clinical findings, and necropsy and laboratory findings.[52] Table 77-1 presents a diagnostic summary of infectious abortions in goats.

The diagnosis in the event of an abortion storm often is not available for several days. It is reasonable to begin treatment of remaining pregnant does with tetracycline in the event that a susceptible infectious agent is involved. One possible protocol is three injections of long-acting tetracycline (LA-200*) at 20 mg/kg at 3-day intervals.[5] Withdrawal time in lactating does should be observed.

Most of the diseases that cause abortion in goats are zoonotic, including chlamydiosis, Q fever, toxoplasmosis, campylobacteriosis, listeriosis, and brucellosis. Owners should be instructed to wear gloves when handling aborted fetuses and to burn or bury any placentas and fetuses not needed for diagnostic efforts. In addition, pasteurization of goat milk for human consumption should be stressed.

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CHAPTER 78

Noninfectious Prenatal Pregnancy Loss in the Doe

WILLIAM BRAUN, JR.

Many noninfectious agents have been named by producers as responsible for pregnancy loss in the goat. Unfortunately, most of this information is anecdotal and not well documented, although it is passed on to other producers as fact. Often an agent or event is implicated because abortion or pregnancy loss follows closely after its application or occurrence. Stress is a good example of this, whether in the form of changing weather patterns or harassment of the does by dogs. Various pharmaceutical and biologic agents have been implicated as causes of abortion when in reality rough handling during administration actually caused the loss.

EARLY EMBRYONIC DEATH

In many cases, it is difficult to differentiate between early embryonic death and conception failure. Fertilization failure and embryonic loss prior to day 15 of gestation both result in return to service at the normal time of the next expected estrus. Most embryonic loss takes place before day 30 of gestation.[1,2] Although fertilization rates may approach 95%, embryonic mortality rate ranges up to 85% with a mean of 20% to 30%.[2] A great amount is known about embryonic death in sheep, but there is a paucity of data for goats. Embryonic losses of 6% to 42% suggest that survival of goat and sheep embryos is similar.[1]

The reason for loss of morphologically normal embryos remains obscure but is probably attributable to physiologic or environmental factors. The selective loss of genetically abnormal embryos is unavoidable. Genetically abnormal embryos contribute a relatively constant, and significant, proportion of observed losses.[2] This loss takes place early in gestation and offers a chance for rebreeding in a relatively short time. In humans, pregnancy occurs in only 18% to 28% of the menstrual cycles.[3] The majority of losses are from embryos with gross chromosomal abnormalities, with trisomy in almost half of these cases.[4] Chromosomal abnormalities, such as centric fusions and translocations, cause an unstable karyotype. Animals are inconsistent with humans, having a lower incidence of chromosomal abnormalities. One study showed that animals had an incidence of chromosomal abnormalities of 8.7%, with mosaicism predominating.[5] The incidence of abnormalities, especially polypoids, increases with aging of the female gametes.

The maternal environment may be unable to support normal development of the embryo or an inappropriate relationship may exist between the embryo and its dam.[4] The maternal environment may be inadequate to support normal gestation either because of an inherent abnormality of the reproductive tract or as a result of an inappropriate hormonal pattern or other outside factors. Progesterone and estrogen determine the proper function of the uterus in preparation for embryonic development. A steroid imbalance may induce asynchrony between the embryo and uterus[2] as shown when embryos are transferred to recipients that are not synchronous with the donor. In these cases, pregnancy is not established and the embryos are lost. The variability of progesterone concentration around estrus and the timing and extent of the luteal rise in progesterone concentration account for a proportion of embryonic deaths.[2]

Ovulation rate seems to play a role in embryo survival in goats. The chance of an embryo surviving in any doe decreases as the ovulation rate rises above two.[2,6] Embryo transfer in the goat has shown that transferring two embryos results in better pregnancy rates and embryo survival than transferring one or three embryos.[7] Prolificacy may be lowered if there is a significant partial loss of multiple embryos with the female remaining pregnant.

Nutrition and stress are two environmental factors that may adversely affect embryo survival. Heat-induced embryonic death is at times an important factor in goat productivity.[2] Does exposed to high ambient temperatures around breeding and during the early cleavage stages experience some embryo loss. Poor nutrition is often implicated when there appears to be no other reason for embryo loss. In sheep, overfeeding or underfeeding may reduce embryo survival.

GENETIC DISORDERS

Habitual abortion is an inherited disorder of some older Angora does. Affected does typically have fine hair coats of high yield, the result of adrenal malfunction. These does conceive normally in their first years, but from the fourth or fifth year begin the habit of aborting. Abortion occurs around day 100 of gestation from continued fetal growth but retardation of placental growth.[8] Prevention is directed at culling aborting does and their earlier offspring.

Does running with rams during the breeding season may breed with the rams without the manager's knowledge. Ram sperm will frequently fertilize goat ova, but the resultant embryo typically dies by 40 to 60 days of gestation. Owners then witness the expulsion of this goat-

sheep hybrid as an abortion. This hybrid has a chromosomal number (57) intermediate to the goat (60) and the sheep (54). Mummification is an occasional sequela.

NUTRITIONAL FACTORS

Nutritional deficiencies, especially energy and protein deficiencies, are suspected of causing some cases of abortion, although their exact mechanism is unknown. Undernourished Angora does may abort after experiencing stress, especially young does that have not been grown out well. During late gestation, inadequate protein and energy levels have been thought to result in abortions, stillbirths, or the birth of weak kids. However, some does near starvation have carried pregnancy to term. Nutritional stress, the withdrawal of concentrates from a basal diet of poor-quality forage, was the reported cause of an abortion storm.[9] Some 62.5% of pregnant does aborted in this report. Abortion from nutritional stress usually occurs between 90 and 120 days of gestation.[9,10] This is the period of accelerating fetal growth, a time when nutrition is crucial to the developing fetus.

Deficiency of energy during late gestation may lead to pregnancy toxemia.[11] The exact etiology of pregnancy toxemia is somewhat vague, but predisposing factors are multiple fetuses that cause the expanding uterus to compress and displace the rumen, and lack of exercise of the pregnant doe resulting in an overconditioned, fat doe prone to fatty liver, stress, and suboptimal feed intake. Undernutrition may be gradual or sudden but leads to hypoglycemia that triggers the onset of pregnancy toxemia.

The disease usually manifests itself during the last 4 weeks of gestation. The initial sign is partial anorexia, with the subsequent loss of energy consumption, causing hypoglycemia. Signs then become progressively more severe. Eventually the doe quits eating and cannot stand unless assisted. The doe then exhibits weight loss, dehydration, forced breathing, and grinding of the teeth. In the final stage, the doe becomes nonresponsive to external stimuli, becomes comatose, and dies. During this later stage she may also abort multiple fetuses.

Diagnosis is based on clinical signs, ketonuria, and hypoglycemia. Common necropsy findings include a yellow-orange fatty liver, enlarged adrenal glands, and multiple fetuses in the uterus. Treatment is based on early recognition of the disease, carbohydrate supplementation, stimulation of gluconeogenesis, and removal of the fetuses. Fluid therapy may be warranted, using dextrose to raise blood glucose levels, bicarbonate or lactate, and balanced electrolytes. Oral supplementation with 4 to 8 oz (120–240 ml) of propylene glycol twice a day may be all that is needed until appetite is restored, if the condition is recognized early. Glucocorticoids have been used to stimulate gluconeogenesis, and they may also cause the premature expulsion of the fetuses. Removal of the fetuses hastens recovery of the dam. This is achieved either by induced abortion or parturition or by cesarean section.

Several mineral deficiencies have been associated with lowered conception rate, embryonic death, and abortion. Deficiencies of iodine, copper, magnesium, and manganese have all been shown to cause abortion or birth of weak kids.[12] Selenium deficiency may cause abortion, and prolonged selenium toxicity is reported to cause abortion.[13] Iodine deficiency may cause stillbirths and delivery of kids with goiter. Copper-deficient does may abort mummified fetuses.

Severe vitamin deficiencies have been blamed for some pregnancy losses. A severe vitamin A deficiency, over a 6-month span, was reported to have caused multiple abortions in a goat herd.[12] This case was associated with a diet limited to dry feed for about 6 months and severe parasitism.

TOXIC PLANTS AND PHARMACEUTICALS

Teratogenic changes or abortion have been associated with several plant species, including *Gutierrezia, Lupinus formosus, Conium maculatum, Nicotiana tabacum,* and *Veratrum californicum.*[12,14] Other plants have been incriminated as possible causes of abortion in goats but data are scarce. Nitrate poisoning may cause plant-induced abortion in pregnant animals. Consumption of stressed nitrate accumulators, such as oat or wheat hay, sorghums, Sudan, rape, pigweed, and others, will change hemoglobin into methemoglobin, causing tissue anoxia that is the probable cause of abortion. Phytoestrogens found in many plants may reduce ovulation rates, reduce fertility, and increase embryonic mortality rate.

Various pharmaceuticals have proved to be abortifacients, or at least their use has been reported to be followed by abortion. A number of anthelmintics are used during late gestation in goats at the time when infectious causes of abortion are most pronounced in their action. The subsequent abortions may be related to the compounds administered, to the handling of the pregnant animals, or to infectious causes. Phenothiazine, given in the last month of gestation, may cause abortions, as might the use of levamisole. Cambendazole and elevated doses of albendazole may be embryotoxic in early pregnancy, but their use later in pregnancy seems to be safe. The use of xylazine or high doses of acetylpromazine in the first half of pregnancy may cause abortion because of their adverse effect on placental perfusion.[15]

The indiscriminate application of hormones in pregnant goats will induce abortion; these have been used purposely for the termination of some pregnancies. The goat is totally dependent on progesterone production by the corpus luteum throughout pregnancy. Little or no progesterone is produced by the caprine placenta. Corticosteroids, estrogens, and prostaglandins given to pregnant goats will induce abortion. Exogenous corticosteroids are not effective in terminating pregnancy until late gestation when the placental pathways have matured. Corticosteroids function by increasing estrogen production by the placenta. Whether from placental production or exogenous administration, estrogens stimulate prostaglandin synthesis and sensitize the myometrium to the effects of oxytocin.[16] Prostaglandin F_2 alpha and its analogues are luteolytic from day 5 of gestation until term. Estrogens and prostaglandins are abortifacient throughout most of gestation.

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CHAPTER 79

Noninfectious Infertility in the Doe

WILLIAM BRAUN, JR.

Infections of the genital tract are seldom causes of infertility in goats,[1] although infectious causes of abortion are important. Postpartum genital tract infection seldom has a residual effect during the following breeding season because of the long period between parturition and breeding, which allows time for recovery. Noninfectious causes of infertility are relatively more common in the goat, and to some extent are a result of their seasonal breeding pattern.

CYCLE LENGTH ABERRATIONS

Aberrations of estrous cycle length adversely affect fertility through early involution of corpus luteum (CL) in cases of short cycles. Such events as puberty, time within the breeding season, introduction of the male, and hormone therapy may result in short estrous cycles that are infertile. Extended cycle length may likewise cause infertility or be a result of infertility, as in cases of early embryonic death.

Puberty

Young does should have reached about 65% of their mature body weight by the time of first breeding. This may not occur during the calendar year of their birth, if they were born late in the kidding season, late spring or early summer. The next year these does are of sufficient

References

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CHAPTER 79

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CYCLE LENGTH ABERRATIONS

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Puberty

Young does should have reached about 65% of their mature body weight by the time of first breeding. This may not occur during the calendar year of their birth, if they were born late in the kidding season, late spring or early summer. The next year these does are of sufficient

size to successfully breed. Does born early in the kidding season, late winter or early spring, should have reached adequate size to start breeding during the fall that same year. Restricted feed intake around the time of puberty may slow development or result in anestrus. Young, lightweight does have an increased frequency of abnormal first cycles, either short or long.[2] Although mating may occur during these first cycles, pregnancy would not be expected to be established. It is prudent to allow young does to cycle two or three times prior to breeding for the first time.

Season

Short estrous cycles are a natural part of the onset of the breeding season.[3,4] These short cycles may be associated with ovulation but followed by a CL that is short-lived. These short cycles have a luteinizing hormone peak of low amplitude, which may be responsible for defective CL formation. Camp and associates,[5] in a study of short estrous cycles, found that 86% of these cycles occurred early in the breeding season and that 55% appeared to be anovulatory. They found that there was no difference in the duration of estrus for short (2.9 ± 0.3 days) versus normal (2.8 ± 0.8 days) cycle groups.

Aberrant cycle length also occurs at the end of the breeding season when does are in transition to the anestrous season. These cycles typically have an extended length. At the end of the breeding season, nonreturn to estrus is not trustworthy to detect pregnancy because long estrous cycles are likely to occur.[2] Some does enter an anestrous period after late breeding and may be mistakenly assumed to be pregnant by their owners.

Male Effect

During the late transition season, novel introduction of a male to a group of females will initiate estrus and cyclicity within the group. Some 97% of females ovulate about 3 days after introduction of the male, but 75% experience short luteal phases of 5 days, followed by a second ovulation with estrus in 89%.[6] Only 68% of the initial ovulations are accompanied by estrus. Fertility at first estrus following exposure to the male is quite variable, but it seems that the shorter the time elapsed from male introduction to first estrus, the lower the fertility.[2] Fertility returns to normal at the second estrus.

Hormone Therapy

The use of hormone therapy during the breeding season has resulted in alteration of the normal cyclic pattern in some goats. Pregnant does given 5 mg prostaglandin F_2 alpha to induce abortion often experience a short cycle following the postabortion estrus.[4] These short estrous cycles are associated with delay and decreased magnitude of the preovulatory luteinizing hormone. Armstrong and colleagues[7] reported that goats experience a high incidence of premature luteal regression following superovulation with pregnant mares' serum gonadotropin. This is usually associated with low embryo recovery. Dexamethasone given in repeated 10 mg daily doses, starting during diestrus, has been shown to extend the estrous cycle by a mean 8.75 ± 0.96 days.[8] Corticosteroid therapy causes a progressive decline in luteinizing hormone response to gonadotropin-releasing hormone and may result in the development of cystic follicles.

Early Embryonic Death

Any condition that results in early embryonic death may result in extended cycle length. The caprine embryo signals its presence between day 16 and 17 of gestation with caprine type I trophoblast interferon.[9] This substance prevents luteolysis and expulsion of the embryo. Removal or death of the embryo after day 15 extends the interestrous interval by about 10 days.

PSEUDOPREGNANCY

Pseudopregnancy, hydrometra, and mucometra are all terms used interchangeably to describe a common occurrence in goats characterized by persistent CL, anestrus, and a variable accumulation of fluid within the uterus. In two large French studies of more than 5000 ultrasonographic scans, the incidence of pseudopregnancy was less than 3%, but some farms experienced more than 5%.[10] This condition may follow a normal breeding or occur without breeding. The accumulation of fluid is often sufficient to result in abdominal enlargement, and the persistent CL causes elevated progesterone concentrations that may remain high for up to 5 months, mimicking normal pregnancy. The spontaneous evacuation of the accumulated fluid is called "cloud burst" by owners. These does may search for a nonexistent kid and some will start lactation.

Some does show no external signs of hydrometra other than a period of anestrus. At the end of this period, a bloody vaginal discharge may be seen. In mated does, this is taken as evidence of abortion. Affected does may show a repetitive pattern of hydrometra or may experience it only once. There is no evidence of an association between hydrometra and cystic ovaries or an obstructed cervix.[1]

The etiology of this condition is unknown. Early embryonic death with persistence of the CL is thought to play a role in the establishment of hydrometra in some recently mated does. The condition also follows estrus during which does are not mated, so early embryonic death would explain only some of the cases. Diagnosis is based on anestrus and ultrasonographic demonstration of an enlarged, thin-walled, fluid-filled uterus without evidence of a fetus, placenta, or placentomes. Progesterone assays would show progesterone concentrations similar to those associated with normal pregnancies. Treatment is with a single dose of prostaglandin F_2 alpha or oxytocin (50 IU IM, twice a day for 4 days).[11] Hydrometra may reoccur after treatment.

CYSTIC OVARIAN DISEASE

Cystic ovarian disease is a clinical diagnosis frequently made in goats. However, little is understood about this condition. A slaughterhouse survey showed that of 1020

tracts examined, 24 (2.4%) had ovarian cysts.[12] In goats, mature graafian follicles measure an average of 10 mm. Follicular structures larger than 12 mm were considered cystic. This same study found 11 cases (1.1%) of parovarian cysts. Unfortunately, no histories were available on any of these goats, so the effect of cysts on cyclicity and fertility is unknown.

The owner's diagnosis of cystic ovaries is based on nymphomaniac behavior of the cycling doe. Abbreviated cycles are typically 5 to 7 days in length, with several in succession. Diagnosis is speculative, based on the history of repeated short cycles. An attempt can be made to examine the ovaries with ultrasonography, but to date no scientific work has been published on this form of diagnosis. Treatment has been extrapolated from that used in cattle, and either human chorionic gonadotropin (250–1000 IU) or gonadotropin-releasing hormone (50–100 μg) is used in an attempt to establish regular cycles. The doe should be bred on the induced estrus.

MISCELLANEOUS CAUSES OF INFERTILITY

Other potential causes of noninfectious infertility in goats include such things as unobserved estrus, anatomic defects, nutrition, and neoplasia. Except for unobserved estrus, these other causes are rare or of minor concern. The doe shows very few outward signs of estrus unless a male is present. In some situations, does will show mounting behavior, but this may be inconsistent. If a teaser animal is not present, many females in estrus will be missed and breeding opportunities lost. Aids to estrus detection include teaser or breeder males, an intersex goat with male behavior, and a buck jar. A buck jar consists of a rag that has been rubbed on the scent glands on the poll of an intact male and kept in a tightly closed jar until needed. The jar is warmed and opened, and the does are allowed to sniff its contents. Estrous does will react to the buck jar as they would to a male.

Anatomic defects may result in infertility or anestrus. The most common of these is the intersex condition. A phenotypic female may behave as a male. Although intersexes are infertile, some breeders use them as teaser animals.

Poor nutrition may result in anestrus or infertility. Starvation or a lack of energy and protein in the diet adversely affects reproduction. Deficiencies of some minerals, including phosphorus, iodine, copper, and manganese, alter cyclicity. Heavy intestinal parasite loads may act synergistically with poor nutrition to cause reproductive failure.

Neoplasia of the reproductive tract is rare in goats, but tumors can result in infertility. Granulosa cell tumors and dysgerminomas of the ovaries have been reported.[1,10] Uterine tumors reported include leiomyosarcoma, adenocarcinoma, and leiomyofibroma.[1,10]

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CHAPTER 80

Inherited Sex Abnormalities in Goats

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The process of sex differentiation is vulnerable to disruptive events emanating from unfavorable maternal environment and abnormal developmental forces created by mutant genes or aberrant chromosomes of the conceptus. These disruptive events may affect the differentiation of the gonads, gonaducts, or the external genitalia, and may lead to the birth of an animal that is difficult to identify as a male or as a female. Such animals with ambiguous sex phenotype are referred to as *intersexes* or *hermaphrodites,* and are further classified as *true* or *pseudohermaphrodites* based on their gonadal morphology. Intersexes whose gonads represent testis and ovary or a combination of testicular and ovarian structures in one or both gonads are referred to as *true hermaphrodites,* whereas those which carry exclusively testes or exclusively ovaries are referred to as male pseudohermaphrodites or female pseudohermaphrodites, respectively. Examples of these types of malformations occur in all domestic animals, tend to recur in families and pedigrees, and represent the most common type of developmental defects detected at birth.[1]

PREVALENCE

Intersexes are relatively common in domestic goats. Even though they occur in all goat breeds, their prevalence varies, depending upon the breed, prolificacy of the line, and the selection practice adopted by breeders.[2] Among the domesticated goats, male pseudohermaphrodites are more frequently seen in dairy breeds than in wool breeds. Their prevalence is extremely low among horned feral goats, whereas they occur at a high rates in lines selected for twinning and/or lack of horns.[2] In the Saanen and Toggenburg breeds raised in the United States, the prevalence of intersexes during the early part of this century was as high as 11% and 6%, respectively, whereas more recent surveys on European dairy goats cross bred to native breeds from different parts of India indicate the prevalence to be under 2%. Among the Shami breed of goats raised in Jordan, 6% of the newborn kids surveyed over a period of 8 years were intersexes.[3] In this breed, selected for the hornless (polled) trait, the most prevalent type (33.6%) of malformation seen was that involving the reproductive system.[3] A majority of goats displaying malformation of the reproductive system are male pseudohermaphrodites, which are sex-reversed genetic females, whereas true hermaphrodites constitute a much smaller percentage of caprine intersexes.

MALE PSEUDOHERMAPHRODITES

The appearance of caprine male pseudohermaphrodites varies from nearly normal females to nearly normal males in their external genitalia, although a majority of these are female-like at birth. As the age of sexual maturity approaches, these animals become larger than their normal female counterparts and acquire a more masculine head with more erect hair on their neck. Their teats are generally small, and the external genitalia are often represented by a short penis or a bulbous clitoris, which becomes enlarged and externally visible at the age of puberty. Hypospadias, represented by narrow slits of varying lengths on the ventral midline due to incomplete fusion of urethral folds, may be present. In some cases the slit may lead into a pouch that, when filled with urine, may overtly resemble a scrotal sac in the normal location, or anywhere between the anal and the urethral openings. The anogenital distance may vary from 3 to 33 cm in length among caprine intersexes depending upon the type of gonads and gonaducts they carry.[4] As a rule, intersexes showing short anogenital distances (3 to 6 cm) have intra-abdominal gonads; poorly developed epididymis, vas deferens, and seminal vesicles; and moderately well developed müllerian duct derivatives, including oviducts, uterine horns, and body of the uterus. Intersexes with greater anogenital distance show exaggerated masculinization of the external and internal genitalia, including scrotal or inguinal gonads, enlarged clitoris or a penis with sheath, and poorly developed body and horns of the uterus.[4] At the age of sexual maturity, intersex goats may start to butt like bucks and act aggressively toward other goats and people and may begin to dribble their urine or stretch out with a concave back and urinate forward between the legs. They also develop the odor characteristic of bucks, and show pronounced male libido in the presence of a normal doe in estrus. The testes are generally intra-abdominal in the normal location of the ovaries, or they may be partially or totally descended. The partially descended testes located in the inguinal region sometimes can be mistaken for udders, especially when the animal reaches the age of puberty and the fat pads become prominent. The testes are generally intra-abdominal, in the normal location of the ovaries, or they may be partially or totally descended. Occasionally, the uterine horn, wrapped around or attached to the ipsilateral testis, herniates through the corresponding inguinal ring into the scrotum. In some cases, the posterior parts of the horns are either connected to a short body of the

uterus that opens into a cervix, or in close association with the vas deferens. The uterine horns are generally filled with mucus and distended in adult life. In some cases the bilateral hysteroceles, resulting from the herniation of the uterine horns into the scrotum, may be mistaken for highly enlarged scrotal testes at the age of puberty.[5]

Intra-abdominal testes of the intersex are invariably smaller than those of normal bucks of corresponding age. The seminiferous tubules of younger intersexes resemble those of immature males but, as they reach the age of sexual maturity, the tubules appear more narrow or irregular in outline and internally lined exclusively by Sertoli cells. In older intersexes the tubular basement membrane is thick and hyalinized, and the seminiferous tubules generally appear atrophic with abundant interstitium consisting of mature and immature Leydig cells and cells resembling fibroblasts. The principal steroid produced by the gonads of caprine intersexes is generally testosterone, although the plasma concentrations of testosterone tend to be lower than those of normal bucks of corresponding age. Because germ cells are always absent in the testes of adult intersexes, these male pseudohermaphrodites are always sterile.[2,4]

TRUE HERMAPHRODITES

True hermaphrodites among caprine intersexes carry bilateral ovotestes or an ovotestis on one side and a testis or an ovary on the other side. Follicles at various stages of development and hormonal activity are seen in the ovaries or on the ovarian structures located on the periphery of the ovotestes. Seminiferous tubules, generally located toward the middle of an ovotestis, are devoid of male germ cells and generally inactive. Adult intersexes of this category with abdominal ovotestes and ovaries often show gonadal hyperplasia or gonadal tumors, or both. These animals generally resemble females more closely in their external and internal genitalia.[2,4]

True hermaphrodites are generally whole-body chimeras. This type of chimerism results from the admixture of cells from male and female embryos at an early stage in embryogenesis or through the fertilization of the second polar body and the ootid by different spermatozoa, one of which carries an X and the other a Y chromosome. This type of intersex may occur in all breeds of goats because accidents of this nature during fertilization or during early embryonic development can take place in any breed. However, the prevalence of this category of intersexes is relatively low in goats, but male pseudohermaphrodites are encountered more frequently in lines of dairy goats selected for twinning and multiple births.[2]

INTERSEXES IN TWINS

Twinning occurs in goats as often as single births, and in selected lines of domestic goats, twins and triplets occur more frequently than singles. Although the birth weight of the kids in twin births is similar to that of single-born normal kids and the incidence of perinatal death is lower in twins compared to that in single births and triplets, the prevalence of intersexes is higher among twins and triplets than in singles.[6] Caprine intersexes among twins and multiple births generally represent the counterparts of freemartins in cattle. As in cattle, caprine freemartinism is caused by the fusion of fetal membranes in twin gestation and the subsequent vascular anastomosis that allows the passage of cells and hormones from a male fetus to the female fetus. Freemartins can be differentiated from the other types of caprine intersexes from their birth history and chromosome makeup. Sometimes the birth status as a twin may not be recognized if one of the twins died in utero. In these cases, confirmation of freemartinism can be obtained by chromosome analysis of peripheral blood cultures. Caprine freemartins would reveal exclusively female cells in solid tissues and the coexistence of male and female cells in blood, even though the proportion of male cells in hematopoietic tissues may often be very low. The external and internal genitalia of caprine freemartins, as in other male pseudohermaphrodites, vary greatly. However, the masculine features are generally more exaggerated and the gonads are partially descended testes devoid of germ cells.[6] Unlike the situation with twinning in cattle, vascular anastomosis does not occur in a majority of caprine twin pregnancies, or it occurs later in gestation after the critical period in ovarian differentiation in the female fetus. As a result, freemartinism is encountered at a much lower rate than that of gestation involving heterosexual twins or triplets in goats. Twinning and multiple births occur in horned and polled goats. Because of this, freemartin-type intersexes can be seen in both types of goats, although polled does, which are more prone to produce twins and triplets, are also more likely to produce freemartins. It would appear that the freemartin-type of male pseudohermaphroditism accounts for approximately 6% of all caprine intersexuality whereas the remaining (over 90%) belong to the category of intersexuality associated with the polled trait.[6]

POLLED INTERSEXES

Intersexes are more abundant among polled (hornless) dairy goats than among their horned counterparts. Polled intersexes are genetic females (XX) and thought to be homozygotes for the polled gene based on their occurrence in proportions matching the genetic expectation among the progeny of polled-to-polled matings. The prevalence of intersexuality in homozygous polled female goats was thought to be due to the closely linked nature of the dominant gene (P) for polled, which is fully penetrant in males and females, with a recessive gene that leads to intersexuality in genetic females (XXPP).[7] According to this hypothesis, the polled does that are fertile are all heterozygotes for the polled locus (XXPp) and all horned does are homozygotes for its recessive allele (XXpp). The mechanism that leads to sex reversal of polled females is not totally understood as yet, although it is clear that the caprine P gene itself, or a segment close to it, in homozygous state has the capacity to initiate testicular induction in XX fetuses, even though they lack the Y chromosome. Furthermore, tests for the male-specific gene sequence referred to as the sex-determining region of the Y chromosome *(SRY),* which is one of the

major genes responsible for testicular induction in mammals, have shown that the polled intersexes are *SRY*-negative.[8] This caprine paradox is explained to some extent by the more recent findings on the polled mutation.

Polled Mutation

Recent studies by scientists in France have shown that the gene for polled is located on the long arm of chromosome 1 of the goat karyotype, at the band designated as 1q43.[9] By haplotype analysis, the segment that carries the polled mutation in goats was localized within a 100-kb segment of DNA homologous to a region on human chromosome 3 (band 3q23).[9] This region of human chromosome 3 harbors the gene for the forkhead transcription factor *(FOXL2),* which, when mutated, causes the disruption of development and maintenance of ovary, eventuating in premature ovarian degeneration and a malformation characterized by drooping eyelids with excessively thick epidermis, referred to as blepharophimosis ptosis epicanthus-inversus syndrome (BPES).[10] A more exhaustive study using positional cloning approach narrowed the location of the caprine mutation to a 25-kb area within this 100-kb segment, and further showed that the mutation responsible for the polled trait-related intersex syndrome (PIS) in goats represents an 11.7-kb deletion.[11] The segment deleted (through PIS mutation) consists of a repetitive sequence-enriched segment of DNA, with long-range regulatory effect on two important genes involved in horn bud development and gonadogenesis. One of these is a noncoding gene located 20 kb away from the deletion and referred to as PIS-regulated transcript 1 *(PISRT1)* because the transcription of this gene is regulated by the PIS locus involved in the deletion. The other gene, located 200 kb away from the PIS locus, is the caprine homologue of the forkhead transcription factor *(FOX L2)* involved in human BPES.[11] Transcription of both these genes is involved in the development of fetal ovaries and horn bud potential, with the levels of transcription in the skin surrounding the future horn bud region depending upon the genotype of the fetus.[11]

PISRT1 *and* FOXL2 *in Gonadogenesis*

In normal goat fetuses free of the polled mutation, *PISRT1* gene transcribes an RNA required for the initial stages of ovarian morphogenesis, but *FOXL2* transcription is also elevated in the mesonephros and in fetal gonads. However, gonadal expression of both these transcripts displays a sex-dependent dimorphism with much higher levels in normal female (XXpp) fetal gonads at 30 and 36 days post coitum (dpc) when the bifunctional gonad is still attached to the mesonephros, compared to that in normal XY male gonads at corresponding stages of growth. Between 40 and 56 dpc, *PISRT1* expression reaches a 5 to 200 fold higher level in normal fetal ovaries relative to that in fetal testes whereas in the latter *PISRT1* expression begins to increase only at 70 dpc and the level increases during gestation, reaches a plateau at birth, and continues to be maintained at high levels throughout sexual maturation and adult life. Transcription of *FOXL2*, which also is elevated in normal fetal ovaries at the early stages of gonadogenesis (30, 36, and 40 dpc), reaches a 30- to 300-fold increase relative to that in fetal testes at these stages. Transcription of *FOXL2* is also slightly increased in fetal testes after 70 dpc, but remains low throughout gestation and postnatal growth period and is absent in adult testes.[11]

PIS Deletion and Sex Reversal

In XXPP goat fetuses (which carry the PIS deletion in both chromosomes of the pair at 1q43), no impact of this deletion is evident during the early stage of gonadogenesis characterized by the formation and growth of the genital ridge.[12] At these stages (up to 36 dpc), the gonads of XXPP fetuses histologically resemble those of normal female fetuses, which are either free of the polled gene (XXpp) or carry the polled gene in heterozygous state (XXPp). However, by 40 dpc, XXPP fetuses begin to display evidence of disrupted ovarian differentiation including elongation of the sex chords and replacement of the coelomic epithelium with the presumptive tunica albuginea, and by 70 dpc the gonads of a majority of XXPP goat fetuses resemble fetal testes while others retain a few oocytes and developing follicles in the cortical region of the ovary.[12]

The gonadal expression of *PISRT1* in XXPP fetuses closely follows the pattern of normal female fetuses at the initial stage of gonadogenesis (before 36 dpc); however, *PISRT1* and *FOXL2* transcription in follicular cell precursors is drastically reduced at 36 dpc.[12] Other evidence of ovarian disruption detectable at this stage is the striking reduction in the transcription of cytochrome P-450 aromatase *(CYP19)* gene, which is responsible for catalyzing the conversion of androgen to estrogen in normal fetal ovaries, even though no departure in ovarian morphology is detected at 36 dpc compared to that of normal XXpp fetuses of corresponding age. By 40 dpc, however, the ovarian cortex is drastically reduced, and the formation of seminiferous tubules and the tunica albuginea is initiated, and the fetal Sertoli cells begin to produce the anti-müllerian hormone (AMH). Unlike normal female fetuses of this age, 40 dpc XXPP goat fetuses generate elevated levels of mRNA for the *SRY*-related high mobility box (SOX9) protein belonging to a family of conserved DNA-binding proteins expressed exclusively in the Sertoli cells. The SOX9-aided secretion of AMH is thought to be the first sign of fetal gonadal function as the testis which, in XXPP fetuses, is already evident by 40 dpc. By 56 dpc, the gonads of XXPP fetuses begin to resemble that of normal male fetuses in morphology as well as in *AMH* and *SOX9* expression pattern, and in their impact on müllerian duct regression. Ovarian germ cells, which in normal (Xxpp) caprine fetuses enter meiosis around 55 dpc, become extremely reduced in XXPP fetuses after 56 dpc, and continue to degenerate throughout gestation. By 70 dpc, signs of masculinization of the gonaduct as epididymis and the initiation of the prostatic buds become evident. Thereafter, the pattern of differentiation of the external genitalia and the expression pattern of other genes follow in the male direction, while the genes expressed in normal fetal ovaries, including *CYP19* and one of the signal protein genes, *WNT4,* required for preventing Leydig cell differentiation in fetal ovaries, remain suppressed.[12]

These events indicate that the functional aspect of the sex reversal process in PPXX fetuses is initiated as early as 36 dpc when the *CYP19* gene is down-regulated, probably by the reduced transcription of *PISRT1* or *FOXL2*. The impact of this reduction is manifested by 40 dpc in the histologic changes taking place in the ovarian cortex, coinciding with the up-regulation of *SOX9* gene expression in the Sertoli cells, suggesting that the follicular cell precursors are the first cell types to be affected by the PIS deletion eventuating in sex reversal.[12]

According to the current concept of mammalian sex determination, the process involves multiple steps, each of which is susceptible to the expression of negative and positive regulator genes located on the autosomes and sex chromosomes, with overt or subtle dosage effects on the expression of other genes.[9] Although all the genes involved in the sex determination cascade have not been identified as yet, some of these active in the genital ridges of both sexes up to the formation of the bipotential gonads, and those specific to, or overexpressed in, the testicular morphogenesis pathway have already been identified.[9] In contrast, only a few genes involved in the morphogenesis of fetal ovary have been identified to date. However, the autosomal genes involved in the male pathway also exist in females and are required for ovarian differentiation. These genes remain suppressed in females by other genes located either on the sex chromosomes or autosomes, such as the autosomal Z locus proposed for human sex differentiation.[13] In normal males, *SRY* codes for the DNA-binding (and bending) protein that functions as a repressor of the autosomal *Z* gene. Because the *Z* locus remains repressed by *SRY* in normal XY males, the expression of male-specific autosomal genes goes uninterrupted, whereas in normal XX females, which lack *SRY,* the *Z* gene is allowed to be expressed and to produce the proteins that would inhibit the genes promoting testis induction.[13] The caprine *PSRT1* is thought to serve as an "anti-testis" gene that inhibits *SOX9* gene expression in fetal ovaries while *FOXL2* ensures the maintenance of fetal ovaries.[12] It has been suggested that a mutation that renders the autosomal *Z* gene inactive *(Z^-)* could allow the male-specific genes of XX individuals to express and, as a result, lead to testis development even in the absence of *SRY.* The PIS deletion, therefore, would appear to be the caprine counterpart of the Z^- mutation implicated in human XX sex reversal because this deletion, through its abrogatory impact on *PISRT1* and *FOXL2* genes, allows testis differentiation-promoting genes to be active in fetal ovaries to cause sex reversal in female goats.

STERILE BUCKS

Testicular Hypoplasia

Prevalence and the leading cause of sterility in bucks vary in goat populations. In general, approximately 33% of sterile bucks also display varying degrees of testicular hypoplasia.[14] Although some of these may be sex-reversed genetic females (freemartins or polled intersexes) erroneously identified as males, other causes of testicular hypoplasia and infertility have also been detected. Testicular hypoplasia is seen in caprine sex chromosome mosaics with XXY/XY cells similar to the partial Klinefelter syndrome in humans.[14] The XXY/XY sex chromosome composition could result from the failure of the sex complements to separate during gametogenesis in either of the parents, followed by the loss of an X chromosome during early cell division in the conceptus. Alternatively, failure of separation of the X chromosome during cell division (nondisjunction) could occur in one of the blastomeres of a male (XY) conceptus. In such situations, the blastomere receiving no X chromosome (due to nondisjunction) is often eliminated early in embryogenesis, and the resulting conceptus displays only the coexistence of XY and XXY cells. Regardless of the mechanism that leads to the mosaic status of the bucks, they may be totally or partially sterile, depending upon the proportion of normal XY cells that happen to populate their seminiferous epithelium.

Sterility in Polled Bucks

Familial type of sterility is more common in polled bucks than in horned bucks.[2] Although the proportions exhibiting sterility vary from 20% to 30%, even among polled bucks, their pedigree indicates that the type of sterility detected is associated with the polled mutation.[4] Unlike the polled female goats that become intersexes, polled bucks show no malformation of the external genitalia at birth and no overt phenotypic abnormality later, other than the lack of horn. These polled bucks undergo normal growth and maturation at puberty, their testes achieve the normal size, and the seminiferous tubules display active and often precocious spermatogenesis. However, they tend to be sterile because of a defect in their gonaduct system resulting in a blockage (stenosis) in the epididymis.[4] The blockage, interrupting the passage of spermatozoa through the duct system, causes retention of spermatozoa in large masses in the lumen of the duct. Occasionally, in older bucks, the seminiferous tubules close to the rete testes degenerate or rupture, causing the release of spermatozoa in the interstitium (extravasation), which often leads to sperm granuloma formation. This type of epididymal stenosis leading to sterility is detected in less than 30% of polled bucks resulting from polled-to-polled matings, but it is believed that the afflicted bucks are homozygotes for the polled gene.[6] Thus, homozygosity for the polled mutation (PIS deletion) appears to be disadvantageous to both sexes: it causes poor differentiation of the androgen-dependent duct system and leads to sterility in the male, and allows the expression of genes in the testicular differentiation pathway leading to the masculinization of the gonad, gonaduct, and external genitalia in the female. The mechanism leading to the interruption of wolffian duct differentiation in XYPP males is not as yet known.

Persistent Müllerian Duct Syndrome

Another malformation of the duct system leading to sterility in the bucks is that referred to as persistent müllerian duct syndrome (PMDS).[15] This defect is considered distinct from caprine intersexuality because affected indi-

viduals are karyotypically males (60XY) with a well-developed penis and bilateral cryptorchidism. Failure of müllerian duct to regress in PMDS males is attributed either to arrested or delayed function of the fetal Sertoli cells, which normally produce the high-molecular-weight glycoprotein dimer, anti-müllerian hormone (AMH) during the differentiation of the embryonic ducts. Müllerian duct regression could also be impeded by the absence of receptors for binding this hormone on the embryonic paramesonephric derivatives. In either case the retention of these embryonic duct systems could lead to cryptorchidism in males because the abdominal müllerian duct derivatives could interfere with the transabdominal testicular descent. This anomaly in goats probably is inherited as an autosomal recessive trait, as in humans and dogs.[15] However, the true identity and frequency of this syndrome in goats may not be resolved, because cases exhibiting male and female internal genitalia with and without bilateral cryptorchidism generally will be included as caprine intersexes. It is also possible that some cases diagnosed as bilateral cryptorchidism in goat breeds may represent PMDS because not all cases are subjected to routine postmortem examination or karyotype analysis. Regardless of the true identity of their affliction, goats with PMDS could develop testicular tumors as they age, as their counterparts in other domestic animal species do.

ELIMINATION OF SEX ANOMALIES

Identification of the cause of an undesirable trait is often the first step leading to its elimination from domestic animals. The discovery of the PIS deletion as the molecular basis of the most common type of sex anomaly in different breeds of dairy goats is an important breakthrough for its relevance to solving the hitherto paradoxical association of two seemingly unrelated attributes in goats and for the light it sheds on the regulation of ovarian differentiation and sex reversal, in general. In the caprine context, polled trait–related intersexuality and epididymal stenosis can be eradicated by avoiding polled-to-polled matings. In contrast, the freemartin type of sex reversal occurs as a result of the destruction of female meiotic germ cells by the cells and hormones communicated by the male co-twin through the shared placenta. The occurrence of this category of sex reversal, associated with twinning and multiple births in goats, is less easy to predict and more difficult to eliminate except by selecting against prolificacy. On the other hand, true hermaphroditism, albeit rare, is likely to remain at the same rate in goat populations because accidents in fertilization or embryogenesis leading to whole-body chimerism and development of bisexual gonads can occur in any breed or type of goats, and the causes of these accidents remain unknown.

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CHAPTER 81

Induced Abortion and Parturition in the Goat

WILLIAM BRAUN, JR.

Elective induction of abortion or parturition is easily accomplished in goats. A functional corpus luteum (CL), the sole source of progesterone, is essential for maintenance of pregnancy throughout gestation in does. The caprine placenta produces little or no progesterone. Anything that interferes with proper function of the CL during gestation will result in termination of pregnancy. By far the most commonly used agent to achieve termination of pregnancy in goats is prostaglandin F_2 alpha ($PGF_{2\alpha}$) or its analogues, but corticosteroids and estrogens have also been employed.

INDICATIONS FOR TERMINATION OF PREGNANCY

The typical reason for a client's request for early termination of pregnancy is mismating. Bucks are clever individuals that are at times almost impossible to keep in their pens. If does are in estrus, bucks will attempt to reach them if at all possible. Mismated does may be too young or small for breeding, held in reserve for breeding at some future date, or scheduled to be bred by artificial insemination or to a different buck. Some older does may have been retired from breeding. Injury or disease that may compromise the life of the doe or the completion of pregnancy may also result in a request for termination of pregnancy.

Induction of parturition has the benefit of allowing prediction of the time of kidding. Many owners prefer this arrangement, as they are able to know in advance when a doe, or group of does, will kid and thus can be prepared for the event. Kiddings can take place when the owners have the time or personnel to attend or assist kiddings. Attended kiddings allow owners to remove the kids from their dams prior to suckling and ingestion of colostrum. This is important in some disease-control programs, especially caprine arthritis-encephalitis and mycoplasma infection, for which the prime mode of transmission to the new generation is through colostrum or milk. Other indications for induced parturition include injuries to the doe during late gestation and impending or early cases of pregnancy toxemia.

INDUCTION PHARMACEUTICALS

Events that lead to normal parturition in goats require functional maturation of the fetal adrenal cortex. Parturition is triggered by activation of the fetal pituitary-adrenal axis.[1,2] Adrenocorticotropic hormone (ACTH) is released by the fetal pituitary, which stimulates release of corticosteroids by the fetal adrenal glands. An increase in fetal corticosteroids stimulates placental estrogen biosynthesis, which in turn stimulates the synthesis and release of $PGF_{2\alpha}$ from the placenta and endometrium. The cascade continues and $PGF_{2\alpha}$ causes luteolysis, which results in a decrease in progesterone. An increase in estrogen and decrease in progesterone stimulate myometrial activity, which is further enhanced by the effects of $PGF_{2\alpha}$, causing a direct effect on the myometrium and stimulating oxytocin release. By mimicking some of these events, abortion or parturition can be artificially induced.

Both ACTH[1,2] and corticosteroids[3,4] have been used for early termination of pregnancy in goats. Infusion of 10 µg ACTH per hour into the fetus on day 126 of gestation resulted in live births on day 131, with placentas expelled within 18 hours after delivery. Daily doses of cortisol acetate (100 mg IM) to does before day 112 and after day 136 resulted in delivery at normal term, but cortisol acetate given on days 113 to 120 resulted in abortion by day 125.[3] Administration of methylprednisolone acetate to does prior to day 84 caused no early termination of pregnancy, but doses of 240 to 270 mg IM given on day 111 or day 125 resulted in abortion in 6 days.[3] In another experiment,[4] nine does were given 16 mg dexamethasone IM on day 144 and all delivered live kids in a mean of 119 ± 29.8 hours, and only one experienced retained fetal membranes 24 hours after delivery.

Exogenous estradiol-17β causes the release of $PGF_{2\alpha}$ and thus induces luteolysis.[5] When infused intravenously into goats pregnant 124 to 142 days, it causes the delivery of fetuses within 54 to 81 hours. All the fetuses died of respiratory failure within 1 hour of delivery. The administration of estradiol benzoate (12 mg IM) to goats at 126 to 138 days of gestation resulted in delivery of live, nonviable fetuses in 58 to 87 hours.[5] In another study, goats given estradiol benzoate (15–25 mg IM) on days 147 and 148 of gestation delivered kids on day 149.[6]

During the breeding season, $PGF_{2\alpha}$ is effective in inducing luteolysis in goats. Luteolysis will occur if the doe is on day 4 to 17 of the estrous cycle when $PGF_{2\alpha}$ is administered. Because the doe is CL-dependent throughout gestation, $PGF_{2\alpha}$ is effective in terminating pregnancy from day 4 until term. Doses as low as 1.25 mg $PGF_{2\alpha}$ have been shown to be effective[7] for luteolysis, as has 0.0385 mg/kg (1.75 mg $PGF_{2\alpha}$/100 lb).[8] One study reported that 15 mg $PGF_{2\alpha}$ given on day 30 or 65 of gestation resulted in abortion at 34 to 54 hours and at 54.5 to 75.5 hours, respectively, after injection.[9] Many does induced to abort with

5 mg or 15 mg $PGF_{2\alpha}$ during the breeding season experienced one or more consecutive interestrous intervals of 2 to 15 days.[10,11] Progesterone concentrations associated with these short cycles suggest failure to form a functional CL or the formation of an atypical CL, either of which was accompanied by a luteinizing hormone surge of decreased magnitude.[10]

The drug of choice for induction of parturition in goats is $PGF_{2\alpha}$ or one of its analogues. When administered IM at doses of 2.5 mg,[12] 5 mg,[12] 15 mg,[9] or 20 mg[4] on days 140 to 144 of gestation, $PGF_{2\alpha}$ will induce parturition and delivery of live kids 29 to 57 hours (average, 43 ± 11.8 hours), 28 to 48 hours (average, 35 ± 8.6 hours), 36 to 56 hours, and 31.5 ± 1.1 hours, respectively, after injection. The higher doses of $PGF_{2\alpha}$ result in a more predictable time of parturition. Treatments are followed by a dramatic decrease in plasma progesterone concentration 24 hours after injection.[4,12] Few cases of retained fetal membranes have been reported with $PGF_{2\alpha}$-induced parturition. Induction of parturition on day 144 did not result in the birth of kids that were smaller than the species average.[12] Owners have reported that some does produce less colostrum when parturition is induced.

MANAGEMENT OF PREGNANCY TERMINATION

The drug of choice for induction of abortion is also $PGF_{2\alpha}$ or one of its analogues. In cases of mismating, the doe should not be treated until 5 to 7 days after breeding, at the earliest, to allow the CL to mature and become receptive to the effects of $PGF_{2\alpha}$. A luteolytic dose of $PGF_{2\alpha}$ is administered IM and the doe is expected to show estrus in 3 to 5 days. If gestational age is 30 days or greater, $PGF_{2\alpha}$ will terminate pregnancy, but the subsequent estrus may be anovulatory, followed by a shortened interestrous interval. Does in which abortion is induced late in the breeding season may not show estrus or cycle again until the next breeding season.

For induction of parturition, gestational age should be at least 144 days. Care must be exercised to ascertain that no unrecorded breedings or errant bucks have caused a miscalculation in gestation length. If the doe is only 123 days instead of 144 days pregnant, induction may produce poorly viable kids. A doe at the correct stage of gestation given 10 to 20 mg $PGF_{2\alpha}$ early one morning can be expected to kid normally the next afternoon, some 30 to 35 hours later. This allows owners to plan the time and day of kidding so that assistance is available or the kids can be removed from their dams prior to suckling.

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CHAPTER 82

Reproductive Health Program

SANDRA L. AYRES

Goats are important livestock animals throughout the world. This is in part related to their ability to survive on suboptimal forage in more arid environments, combined with their smaller size, shorter gestation period, and increased litter size, particularly when compared to bovids. The Food and Agriculture Organization (FAO) figures for 2004 estimate the total goat population in the world at approximately 780 million animals.[1] Even in the United States, where goat production is lower than in other parts of the world, the 2004 kid crop was estimated to be 1.67 million animals.[2] Goats in the United States were used primarily to produce meat (1.97 million animals), goat's milk (283,500 animals), and fiber (274,000 animals).[2]

The success of any goat production operation depends upon optimizing reproductive health. Although the basic requirements for good reproductive health are similar in all types of goat operations, there are some differences in the reproductive demands and husbandry practices, particularly for dairy animals as compared to goats raised for meat and fiber. Areas of importance for reproduction in goats include how the herd is housed and pastured, the nutritional requirements of the herd relative to type of production and breed, disease and parasite control, reproduction program, and special requirements for dairy operations.

HOUSING AND PASTURE

Most goats reared for meat and fiber are housed on pasture or range. Some access to protective shelter or housing is preferable, particularly during the kidding season. Goats are unlike sheep; whereas newborn lambs immediately follow the dam, does naturally hide their kids for the first few days after birth, thus making newborn kids vulnerable to predation.[3] This also means that pastures should be carefully checked for young kids if moving the herd during the kidding season.

Unless breeding is being timed or synchronized, it is common to run the bucks with the does, allowing breeding to occur throughout the breeding season.[4] A stocking rate of 1 buck to 50 does is usually suggested, although stocking rates as high as 1 buck to 250 does have been reported.[5]

Dairy operations usually keep goats housed indoors, sometimes with access to pasture. If bucks are kept on the premise, they are separated from the does so that estrous synchronization and breeding can be controlled. Bucks that are kept isolated from females can be used to synchronize the does via a pheromonal phenomenon known as the "male effect" or "buck effect."[6–8] Some goat dairies do not keep bucks on the premises, but rely upon artificial insemination (AI) for breeding and to increase genetic variability within the herd.[9]

Bucks that live together establish a dominance hierarchy. Fighting among bucks to establish this hierarchy can be severe, particularly during the breeding season. In addition to acquiring physical injuries, bucks housed with females or near females during the breeding season can lose significant amounts of weight by fighting among themselves rather than eating. Dominance behavior in group-housed bucks usually takes the form of rearing and fighting, but can also include homosexual behavior during the breeding season.[10] Although this behavior has the potential to spread disease, venereal disease among bucks is not as great a concern as it is in rams where *Brucella ovis* is spread by this behavior.[5] Subordinate bucks can have their breeding potential reduced by dominant bucks. If more than one buck is housed with does during the breeding season, the dominant buck can physically interfere with other bucks attempting to breed, a phenomenon sometimes called the "dominance effect." Even when separated from dominant bucks, subordinate bucks can be psychologically intimidated from breeding does if the dominant male is close by ("audience effect"). Subordinate bucks can be induced to breed by putting them in a pen with females situated at a distance away from the dominant buck, or by housing the male with a group of smaller or younger bucks (personal communication, Dr. William Gavin, Stephen Blash, GTC Biotherapeutics).

Does will also form dominance hierarchies. If a group contains both horned and de-horned or polled does, the does with horns will most often become the alpha goats. Dominant does will sometimes physically attempt to keep the most subordinate does from feeding. This is particularly true in situations in which hay and grain is delivered in stationary feeders that can be "patrolled" or defended by the alpha doe. In some situations it may be necessary to separate the alpha does from the subordinate does during feeding to ensure that subordinate does get their fair share of the feed, and that the alpha does eat rather than "patrol" the feeder.[11]

Because of the young age at which goats can attain puberty, it is important to separate young bucks from does at an early age.[12] Bucks as young as $3^1/_2$ months of age have been known to impregnate does, including their siblings or dam.

NUTRITION

Good nutrition is necessary for goats to successfully reproduce. It is suggested that goats attain two thirds of

their adult weight before being used for breeding.[5] Breeding young does that are very small in stature can result in dystocias, particularly in small does carrying large singletons. It is also possible to stunt the growth of the doe by breeding at too young an age.

It is important to understand the nutritional requirements of the breeds being kept to prevent overconditioning as well as underconditioning of animals. Meat breeds such as Boers or Spanish goats can convert feed to muscle mass at a far greater efficiency than most dairy breeds (e.g., Saanens, Alpines) and therefore require less feed for maintenance. This can be problematic when different breeds are housed together, or when dairy maintenance regimens that include grain are used for meat breeds.

Meat and fiber goats can obtain adequate nutrition when reared on good quality pasture. Goats are selective browsers and usually avoid eating toxic plants, but problems can arise if the pasture is marginal, or if vegetation is sparse during dry periods. Goats will then consume forage that can result in reproductive problems. A study in Texas showed that bucks grazing pastures containing plants with high levels of dietary phenolic amines such as those found in shrubs of the genus *Acacia* had a significant reductions in serum testosterone, scrotal circumference, and semen volume.[13] Other toxic plants that can lead to reproductive problems include *Lupinus formosus, Conium macilatum, Nicotiana tabacum,* and V*eratrum californicum*.[3]

Breeding bucks need to be in good body condition. A body score of 3.0 to 3.5 has been suggested.[12] It is important that good body condition be attained prior to the breeding season, as bucks tend to lose weight during this time, particularly when group-housed. Feeding regimens should be changed with care, as there can be problems associated with increasing nutritional planes in bucks. Such increases in feeds are most commonly seen in bucks or wethers being raised for the meat market when additional feeds are used to increase muscle mass or decrease the time needed to attain market weight. A variety of substances have been added to a basic grass diet to increase weight gains including grains, and by-products such as soy hulls, corn gluten, wheat middlings, etc.[14] Diets that result in high protein can lead to ulcerative posthitis, a condition related to production of high levels of ammonia in the urine that, if severe, can reduce a buck's willingness to breed.[3,5] Diets that are high in phosphorus can change the calcium-to-phosphorus ratio to less than 1.5:1 to 2.0:1 and result in the formation of struvite uroliths within the urethra and bladder. Feeds that are high in calcium, such as alfalfa or subterranean clover, can also contribute to the formation of uroliths. Access to clean water, and addition of salt and ammonium chloride to the diet can help prevent the production of uroliths in males.[12]

Feed regimens are important reproductively for females as well as for males. Does should be in good condition prior to breeding. Body scores of 2.5 to 3.5 (out of 5) have been suggested as optimal.[12] Like ewes, does can respond to increased nutrition just prior to the breeding season (flushing) with an increase in the average number of ovulations. But overfeeding of does, particularly dry does, is to be avoided. Overfeeding of grain can result in obese females that exhibit high body condition scores and are more prone to dystocias, in part related to fat deposits in the area of the birth canal. Overconditioned females are also predisposed to develop pregnancy toxemia.[15,16] Inadequate nutrition can also interfere with reproduction. Low nutrition can result in poor ovulation rates or cessation of cycling. When cycling does were fed a restricted diet (30% of requirement) they did not ovulate in response to an estrous synchronization regimen, exhibiting a reduced frequency of luteinizing hormone (LH) pulses.[17]

DISEASES AND PARASITE CONTROL

Diseases can affect reproductive health in a number of ways. Most diseases of goats, such as pneumonia, have at least an indirect effect on reproductive health through reduction of appetite, production of fever, loss of weight, or reduction of ambulation. A sick animal is less likely to breed and to carry pregnancy to term. Chronic illnesses such as Johne's disease, caseous lymphadenitis, and caprine arthritis-encephalitis (CAE) can sometimes have insidious effects on reproductive health. Caprine arthritis-encephalitis is a slow lentivirus of particular concern to the dairy goat industry. Early CAE can negatively impact milk production while producing few clinical signs.[18] The prion diseases, including scrapie and the transmissible spongiform encephalopathies, not only cause a wasting disease but are also of concern for public health.[19]

The set of diseases that most directly impacts goat reproductive health consists of those that cause abortion. Abortion occurs sporadically in goats, but endemic infectious abortion has the greatest economic impact. Many infectious agents have been incriminated in goat abortions, but *Chlamydia psittaci, Toxoplasma gondii, Coxiella burnetii,* and *Mycoplasma mycoides* are the most commonly diagnosed.[16] Ovine chlamydial vaccines provide moderate protection against abortion. Toxoplasmosis is best prevented by controlling cat populations, especially by preventing reproduction, and also by control of rodents. Ionophores fed to dry does may decrease abortion in exposed individuals. The control of *Mycoplasma* infection requires a multifactorial approach with detection of carriers, control of milk transmission, and culling of identified carriers.

Chlamydia is the most common cause of infectious abortion in high-density confinement goat dairies. Does exposed any time (including neonates) prior to day 90 of gestation are susceptible to abortion of that pregnancy, while does exposed from day 100 of gestation onward are at risk of aborting during the next pregnancy due to the long incubation period required to develop necrotic plancentitis that leads to abortion. Although *Chlamydia* infection is traditionally associated with last semester abortion, experimental infection around day 60 of gestation, when the organism first crosses the placenta, may result in fetal death with resorption or abortion.[16]

Disease can be controlled by good husbandry practices and vaccination programs. If animals are housed indoors, particular attention should be paid to keeping animal

densities from becoming too high, having a good waste removal program, and having adequate ventilation. Vaccination programs vary depending upon region and country, but usually include vaccines for *Clostridium perfringens* types C and D, and *Clostridium tetani*.[3,12] If rabies, chlamydiosis, or leptospirosis is endemic, appropriate vaccines are also included. In areas deficient in soil selenium, vitamin E and selenium are administered to prevent white muscle disease.[20] It is important to schedule vaccinations to pregnant does 3 to 4 weeks prior to delivery to ensure adequate antibody production in colostrum.[3,12]

Large parasite loads can induce chronic weight loss, diarrhea, and anemia in animals and thereby reduce reproductive potential.[21] This is of increased concern with the development of strains of parasites such as *Haemonchus contortus* that have become resistant to anthelmintic treatments.[22] Understanding the ways in which choice of anthelmintic drugs and timing of treatments for parasite control contribute to selection for resistance (e.g., frequent use of the same class of anthelmintic) are important to prevent the development of resistant parasites.[23] For animals on pasture, knowledge of parasite cycles combined with schedules of pasture rotation may be effective in helping reduce parasite loads.[23] Investigations are ongoing to find genetic markers for goats that are more resistant to parasite infection, as well as ways to increase natural resistance. For example, it has been suggested that high-producing dairy goats may benefit from supplemental protein in the diet as a way to increase resistance to nematode infection.[24]

REPRODUCTIVE PROGRAM

It is important to maximize fertility in both does and bucks. In females, constraints to maximizing reproductive potential include failure to conceive, early embryonic death, endometritis, pregnancy toxemia, short estrous cycles, ovarian disorders, hydrometra, and abortion. Mucometra, pyometra, and mummified or macerated fetuses are conditions that may result in failure of the dam to show heat or conceive. Real-time ultrasonographic examination and vaginal speculum examination are essential to scrutinize does with apparent reproductive failure.[5] Blood concentrations of hormones (progesterone, estrogen, testosterone), laparoscopy, or laparotomy can also be used.[5] Does with a history of reproductive failure have a poor prognosis for recovery, and a high cost is associated with diagnosis and treatment.

In males, the overall breeding soundness of the buck is often not considered because in many operations there is a high buck-to-doe ratio. The breeding potential of the male is of concern in synchronized breeding schemes that require maximum breeding soundness, or when a valuable buck is providing stud services, or having semen frozen. In any case, breeding soundness examinations (BSEs) should be performed on bucks that are used in breeding programs. Such examinations are important not only to confirm the potential fertility of a buck (scrotal examination, sperm morphology and motility) but also to identify conformational and physical problems that could affect fertility.[5] In addition, buck fertility may be affected by testicular atrophy associated with increased age, poor libido, sperm granulomas in the epididymis, systemic disease, high environmental temperatures, and genetic factors.

In both sexes, the intersex condition related to the polled gene can lead to reduced fertility or infertility.[25] This should be a consideration in young animals in herds containing polled animals, and can be managed by using horned bucks for breeding. Does 6 to 10 months of age that fail to breed with their cohort should be culled unless there are compelling reasons to maintain an individual animal.

In many operations it is important to synchronize the breeding of animals or to breed animals outside the breeding season. This is of particular importance for timed artificial insemination, for dairy operations that want to maintain a year-round supply of milk, as well as for meat operations that want to take advantage of holiday demands for meat. The most common methods of synchronization involve the "buck or male effect," hormones, or manipulation of light. The "buck effect" is most commonly used to synchronize does at the beginning of the breeding season.[26] The scent of the male induces ovulations within 5 days of introduction. The first heat is often not fertile, as it is accompanied by premature luteal regression and a short cycle, but the heat following the short cycle is fertile. If males are housed at a distance from the females, the "buck effect" can also be used to synchronize does during the breeding season. There has also been success in synchronizing does by replacing the original male in the herd with a novel male.

Hormones are commonly used by the dairy industry and in research to precisely control the estrous cycle and to induce cycling outside the breeding season. Hormones used include prostaglandin F2 alpha ($PGF_{2\alpha}$) during the breeding season, and combinations of progesterone (sponges, controlled internal drug releasing devices [CIDRs], subcutaneous implants, injections, and oral supplements [melengesterol acetate]) with follicle-stimulating hormone (FSH) or equine chorionic gonadotropin (eCG) and pregnant mare serum gonadotropin PMSG) during both the breeding and nonbreeding seasons.[27,28] Regulations vary by country as to the use of these hormones in animals used for milk and meat. For example, the current sponges, CIDRs, and subcutaneous implants on the market have not been approved for commercial use in the United States, leading producers to seek alternative methods to cycle animals.[29]

Manipulation of light cycles is also used to synchronize does and induce cycling out of season.[28] Many programs incorporate 6 weeks of long light (>12 hours per day) followed by a period of shorter light. These programs work best with goats that can be housed indoors. An example of this type of light management cycle is shown in Figure 82-1.

Another important reproduction management technique is to determine those animals that have been bred so that open does can be separated or culled, and the pregnant animals can potentially be segregated according

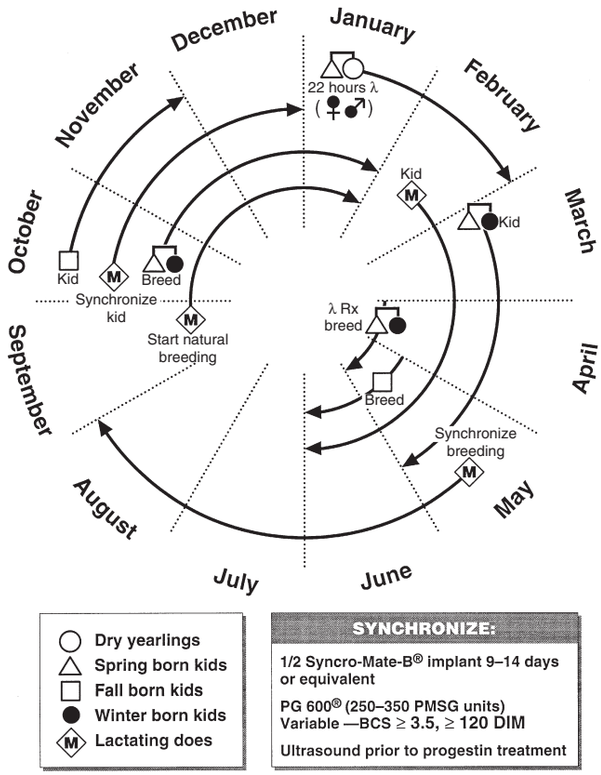

Fig. 82-1 A model to minimize seasonality of milk production in a commercial goat dairy. λ = exposure to lights.

to the number of kids the does are carrying. One method of pregnancy detection involves looking for a return to estrus 18 to 21 days after breeding. In this case it is important that the dates of breeding are known. Another method is to ultrasound the herd approximately 40 to 60 days after breeding. B-mode live ultrasound can detect pregnancy at 45 days with approximately 98% accuracy.[5] Using ultrasound, it is possible to distinguish does carrying singletons from does carrying multiple kids. In addition, ultrasound can be used to determine viability of kids during pregnancy and to distinguish pregnancy from hydrometras.

Advanced or assisted reproductive techniques are now being used in some goat operations.[28] AI has been used, particularly in the goat dairy industry, to produce pregnancies without keeping a male on the premise.[9] With the ability to synchronize the estrous cycle and induce superovulation, it is possible to perform embryo transfer (ET).[27] The difference between performing ET in horses and cattle and goats is the necessity to use a laparoscope or surgical approach for collection and transfer of embryos. Trans-surgical collection has been attempted but appears to be operator-dependent in regard to success.

For all aspects of reproductive management, it is imperative to keep good records. Individual health records assure that data is kept on vaccination and herd health programs as well as affording a way to track infectious disease trends, especially those related to abortion. Records should be kept for all matings so that success rates can be calculated for both males and females. Animals with poor breeding records should be removed from the breeding herd. In addition, animals born with congenital anomalies should be culled and if the anomaly is genetic, the dam and sire should be removed from the breeding herd as well as other related animals.

SPECIAL CONCERNS FOR DAIRY OPERATIONS

In goat dairies, the necessity to produce an even milk supply throughout the year combined with intense housing requirements means that these operations need different husbandry and reproductive programs when compared to meat and fiber operations.

Out-of-season breeding of does is required to maintain a viable commercial goat dairy. Attention to detail, records, and planning are essential for success. About one third of the does should kid from October through December to maintain adequate milk production. Dry stock can be induced to breed out-of-season using exposure of bucks and does to increased light for 60 days in January and February. Estrous cycles should begin 4 to 6 weeks later. Does are loosely synchronized and often have more than one estrous cycle. Although lactating does can be exposed to light treatment, the response is more predictable if synthetic progestins and pregnant mares' serum gonadotropin (PMSG) are utilized. The dosage of PMSG is adjusted based on month of breeding, geographic factors, and reproductive history of the dairy. A single synchronized estrus results, with expected conception rates of 65% to 85% (breeding May 1 to August 31). Doe kids born from these breedings will have one or two heat cycles in the spring, which results in kidding at 10 to 15 months of age. In selection of mature milking does for estrus synchronization, one should choose does with body condition scores of 3 or higher (on a scale of 1 to 5) and at least 120 days in milk. Does with a history of reproductive problems or concurrent disease are best not used in estrus synchronization programs. When planning for does to freshen, it is safest to estimate that only 65% of synchronized does will kid to that mating. If more than 20 to 30 does are synchronized, extra help is needed, because hand-mating is required to maximize success. Artificial insemination can be used in estrus synchronization programs if high-quality frozen semen (determined by post-thaw microscopic examination) is available; however, artificial insemination is an additional variable affecting conception rate.[9] All does exposed to bucks should be examined by ultrasonography 35 to 45 days after breeding and again at 60 to 70 days to confirm pregnancy (in order not to miss the chance of breeding an open doe) and then again when they are dried off. Does that develop hydrometra should be treated with prostaglandin as soon as the condition is detected, rechecked in 10 to 14 days, and re-treated if fluid is still present in the uterus. Does that kid in the fall to early winter may have estrous cycles after kidding and develop hydrometra. Ultrasonographic examination of these does in January to March allows treatment prior to breeding. It is important for pregnant does to have a 60-day dry period if they are expected to reach maximum milk pro-

duction. A truncated dry period may result in production losses of at least 20% to 30%. Open does detected at the "due to dry date" can continue to be milked if production is adequate. Generally, these does increase their milk production for a period, or yield a fairly constant amount during their extended lactation.

Of special concern to dairy operations is the disease caprine arthritis-encephalitis (CAE). The three forms of this disease are a fatal encephalitis affecting young kids, a condition known as "hard udder" affecting milking does, and an arthritic form that affects joints, particularly the carpal joints. CAE is a slow lentivirus that is usually transmitted vertically from mother to young, but can also be transmitted horizontally. Detection can be difficult, as the disease often does not manifest until the animal is 18 months of age or older.[30] The disease is most commonly transmitted via untreated colostrum and unpasteurized milk fed to kids, and can possibly be transmitted in utero.[31,32] Steps can be taken in a dairy operation to eliminate CAE in the herd. The herd should be tested for CAE, and those animals that test positive should be separated from the rest of the herd or culled. Births should be attended, particularly of CAE positive does, and the kids removed from the dam immediately at birth so the kids do not suckle. There has been some recent success using a "goat-side" progesterone assays (enzyme immunoassay) to predict the 24-hour window in which the doe will give birth.[33] Kids should be fed colostrum heat-treated at 56°C for 1 hour, and fed pasteurized milk. Kids from positive mothers should not be mixed with kids from negative mothers. After 6 months of age, kids should be tested for CAE, and only negative animals used for breeding. Positive kids should be culled. Because there is evidence that CAE can be found in oviductal cells,[34,35] granulosa cells,[36] and in embryo flush medium,[31,35] care must be taken when performing embryo transfers that embryos are produced from animals testing negative for CAE. Although animals have been known to seroconvert,[37] particularly when mixed with animals that are seropositive for CAE, regular testing schedules and careful rearing of young combined with culling of positive animals or closing the herd has resulted in eradication of the disease from some facilities.[18,32,38]

Dairy operations differ from other goat operations in the increase in labor needed to milk animals and rear young, as well as dealing with milking-related diseases such as milk fever and mastitis. Many large dairy facilities milk their animals two to three times per day, although recent work indicates that once-a-day milking can be used without impacting udder health, and may be effective for small operations by offsetting the reduction in milk volume with a reduction in labor needed.[39] Most dairies remove the kids from the dam at birth or soon after as part of disease control programs and to prevent bonding between the doe and kid, although programs have been designed that allow kids to suckle the dam while still being milked once a day.[40] Kids need to be fed heat-treated colostrum within the first 24 hours after birth and can then be fed pasteurized goat milk, goat milk replacer, or even cow milk twice a day for 7 to 9 weeks. In milking does mastitis is a concern.[41] A number of agents can produce mastitis, with *Staphylococcus aureus* frequently reported for clinical mastitis, and coagulase-negative staphylococci are often reported in subclinical mastitis.[42] Unlike cattle, in which somatic cell count (SCC) can be used to monitor mastitis in a herd, SCC in goats is a less reliable indicator of mastitis because the cell level is influenced by many factors including age of the animal, stage of lactation and level of milk production, breed, infection with CAE, and estrus.[43] Mastitis in the herd can be reduced by careful attention to sanitation and proper functioning of milking equipment, proper cleaning of the udder, good diagnosis, and treatment of mastitis with culling where appropriate, and awareness of the possibility of genetic components for susceptibility and resistance to mastitis.[42,43]

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CHAPTER 83

Reproductive Biotechnologies in the Goat

GABRIELA FLORES-FOXWORTH

In recent decades, scientists have contributed to develop powerful biotechnologies such as estrus synchronization, superovulation, artificial insemination, embryo collection and transfer, in vitro embryo production, cryopreservation, a variety of embryo micromanipulation procedures, and nuclear transfer. The development of these technologies enables the manipulation of animal reproduction to rapidly propagate superior or desired animals and genes, the genetic modification of animals to improve their production quality, and the production of medically significant products. The goat's diversified commercial value, size, relatively short gestation period (when compared to the cow), and popular demand make it a convenient species for new technologic research and application.

EMBRYO COLLECTION AND TRANSFER

Embryo collection and transfer are very valuable techniques for the rapid dissemination of desirable genetics. Commercial application of this technology in goats was somewhat limited in the United States until the importation of the South African Boer goat. Embryo transfer programs in goats yield highly variable results. The major contribution to this variability is the unpredictable response to superovulation. Incorporation of recent protocols to control follicular wave dynamics in the bovine permits the initiation of superovulatory treatments at a self-appointed time, reducing the variability of the response, and this is applicable in goats. Goats are also extremely sensitive to both nutritional and physical stress; this must be taken into account when handling goats in any embryo transfer program. Recipients should be healthy, well-grown animals that have been on location for preferably at least 4 months. Otherwise, relatively high percentages (up to 25–30%) of treated animals will not be usable on the intended day of transfer for reasons such as failure of ovulation or early luteal regression.

Synchronization and Superovulation

Many combinations of treatments for the purposes of embryo collection and transfer are available. Estrus can be synchronized by the administration of progestogens such as progesterone implants or synthetic progestins (flurogestone acetate, FGA; medroxyprogesterone acetate, MAP) given either orally or by the insertion of a vaginal sponge. The most widely used synchronization device for goats is the control internal drug release progesterone implant (CIDR-G, 0.3 g progesterone, Eazi Breed, InterAg, New Zealand) which is inserted in the goat's vagina using a special applicator. Most traditional schemes consist of a long progestagen (12–18 days) treatment; recent protocols use a shorter progestagen treatment (5–9 days) accompanied by a prostaglandin F2 alpha ($PGF_{2\alpha}$) analogue injection.[1] During the breeding season an analogue of $PGF_{2\alpha}$ is commonly given at the time of device insertion to destroy the corpus luteum and obtain a greater percentage of estrus synchronization. The method for synchronization of estrus is the same for both the donor and the recipients. Owing to the hormonal treatment administered to donors, estrus occurs sooner than in the recipients; therefore implants should be removed from recipients 12 hours prior to removal of the progesterone or progestin source from donors. Some synchronization programs recommend the replacement of the progesterone or progestin source midway through a long synchronization regimen to maintain high levels of progesterone during gonadotropin therapy.

For the induction of superovulation of donor goats, pituitary extracts of follicle-stimulating hormone (FSH) and pregnant mares' serum gonadotropin (PMSG; also called equine chorionic gonadotropin, eCG) are the gonadotropins most used. Commercially available FSH products are: Folltropin V (Vetrepharm, Ontario, Canada), Ovagen (ICP, Auckland, NZ) and Super-Ov, (Ausa International, Tyler, TX). Several protocols can be used for superovulating goats, most commonly the injection of multiple doses of FSH on the last 3 to 4 days of the progestagen treatment. Due to the short half-life of the FSH molecule, it is traditionally administered every 12 hours. One example is the twice-a-day injection of a series of decreasing doses of FSH (5, 5; 3, 3; and 2, 2 mg per injection), with a total dose of 20 mg, with the next to last injection accompanied by progesterone removal and an injection of 150 μg of a $PGF_{2\alpha}$ analogue. Goats display behavioral estrus at approximately 24 hours after implant removal. Folltropin V can also be administered in decreasing doses (3.6, 3.6; 3.2, 3.2; 2.4, 2.4; and 1.6 mg) with a 17-day progestagen treatment; a simpler protocol consists of six injections at a level dose given every 12 hours (total dose equivalent to 20 mg NIH-FSH-P1).[2] An average of 17 ovulations have been obtained per doe using this protocol.[3] Super-Ov is a highly purified preparation of FSH that is marketed in units of FSH activity per milliliter rather than in milligrams per milliliter. Doses are calculated as milliliters of the reconstituted product; a 10-ml vial contains 75 units of activity. One

commercially used regimen of Super-Ov in goats is a 4-day declining dose (1.0, 1.0; 0.8, 0.8; 0.6, 0.6; and 0.4, 0.4 ml) that is reduced by 0.2 ml per injection for young does. Another example is a level dose of 0.7 ml bid for 4 days. Superovulatory treatments using Ovagen (which has a consistent level of LH activity) consist of eight IM injections of 1.25 ml given twice daily starting 60 hours before progestagen removal. The response to this protocol has been reported to be of 14.3 ± 0.5 corpora lutea, 11.3 ± 0.5 recovered embryos, and 6.8 ± 0.4 viable embryos per goat.[4]

Recently the use of estrogens at the time of progesterone implant insertion and removal have improved superovulation responses. The ovarian response in terms of number of corpora lutea has been shown to be correlated to the number of dominant follicles at the onset of gonadotropin treatment. A higher number of 2- to 6-mm follicles tended to be associated with a lower embryo recovery rate. Estrogens (such as estradiol benzoate, EB) have been shown in the bovine to induce regression of those dominant follicles, which are likely to be immature or in the process of atresia, followed by a synchronous follicular wave emergence (4–5 days after EB injection). This wave generally coincides with the time of the administration of the FSH treatment. Therefore, at the time of FSH injections a uniform cohort of follicles is recruited and no dominant follicles are present.[2,4,5]

When PMSG is used, a single injection 24 to 48 hours prior to implant removal is administered to the donor doe. Doses of PMSG range from 500 IU to 1500 IU, depending on breed, age, and size of doe and previous responses to treatment. One of the disadvantages of using PMSG is the tendency to produce persistent follicles that interfere with fertilization and overall embryo collection rates.[6]

It is important to note that there is a high variability in response rate to superovulatory treatments between animals, and factors such as follicular status, nutrition, lactation, seasonality, and management, among others, have an influence on the superovulatory response.

Estrus Detection and Breeding

Estrus is detected in donor does by observation of mating; in recipients estrus should be detected using vasectomized bucks or androgenized females. The time from beginning of estrus to ovulation in goats is approximately 32 to 36 hours. If laparoscopic artificial insemination is to be performed, does should be inseminated 12 and 24 hours after the animals were first seen in estrus or 45 to 50 hours after implant withdrawal.[7]

LAPAROSCOPIC INSEMINATION

Laparoscopic artificial insemination allows semen to be placed directly into the lumen of the uterine horns considerably closer to the site of ovum fertilization. High fertilization rates (80%)[8] can be achieved with this technique, compared with transcervical insemination (50%) using fresh diluted semen.[7]

The goat is secured in a laparoscopic cradle and is tilted head down at an angle of 40 degrees or more. Pneumoperitoneum is produced by introducing CO_2 through a Verres needle inserted transabdominally. This separates the abdominal wall from the viscera, clearing the field for manipulation of the reproductive tract. The trocar and cannula for the laparoscope (5–7 mm diameter) are inserted into the peritoneal cavity approximately 10 cm cranial to the udder and to the left of the midline. The secondary trocar and cannula (5 mm diameter) are inserted to the right of the midline. The laparoscope is inserted through the cannula and the uterus is located just cranial to the bladder. An assistant prepares the inseminating gun (IMV International Corp-USA, Minneapolis, MN) or pipette (glass) and hands it to the operator. The operator guides the tip of the inseminating gun toward one uterine horn. Care should be taken not to perforate any viscera with the sharp needle or tip of the inseminating device. While visualizing through the laparoscope, the site of insemination is selected along the major curvature of the uterine horn and the needle is stabbed through the myometrium and into the lumen in a perpendicular position (Fig. 83-1). Semen should be deposited with no resistance and no swelling of the uterine wall should be seen, indicating that the needle is correctly positioned in the lumen of the uterine horn. The inseminating gun is removed, and the operation is repeated on the other uterine horn. The instruments are removed and the abdominal wounds are sutured. In most cases the dose of semen contained in one straw (40–100 million motile sperm cells) is divided in both uterine horns. If fresh semen is being used, reports indicate that the effective dose is about 20×10^6 for laparoscopic AI, and 100×10^6 for cervical AI.[8]

Anesthesia: Animal and Equipment Preparation

Animals are taken off feed 18 to 24 hours prior to any surgical procedure. The animals are anesthetized with an anesthetic of choice. A review of commonly used anesthetic regimens is available.[9] In this author's laboratory, a combination of xylazine and ketamine (0.15 mg/kg xylazine and 3.6 mg/kg ketamine) is used intravenously to perform laparoscopic embryo transfer into recipients or for induction when gas anesthesia is used for embryo collection. When gas anesthesia is not available, a combination of xylazine and ketamine can be used alone (0.22 mg/kg xylazine and 5.5 mg/kg ketamine), with half the dose administered IV and half IM to maintain anesthesia during embryo collection. If necessary, animals can be re-dosed with 1.9 mg/kg ketamine IV. Because of profound effects of higher doses of xylazine (>0.15 mg/kg) on cardiovascular and respiratory parameters in sheep, it has been recommended that dosing in goats also be as conservative as possible.[9] The effects of xylazine can be reduced postoperatively by administration of an antagonist such as yohimbine (0.11 mg/kg IV). However, if ketamine levels are still elevated at this time, reversal of xylazine will result in tremors. A combination of tiletamine and zolazepam (Telazol, Fort Dodge Laboratories, Fort Dodge, IA) at a dosage of 5.5 mg/kg IV has been reported to provide at least 1 hour of anesthesia.[9] In our experience, when goats are elevated in a laparoscopic

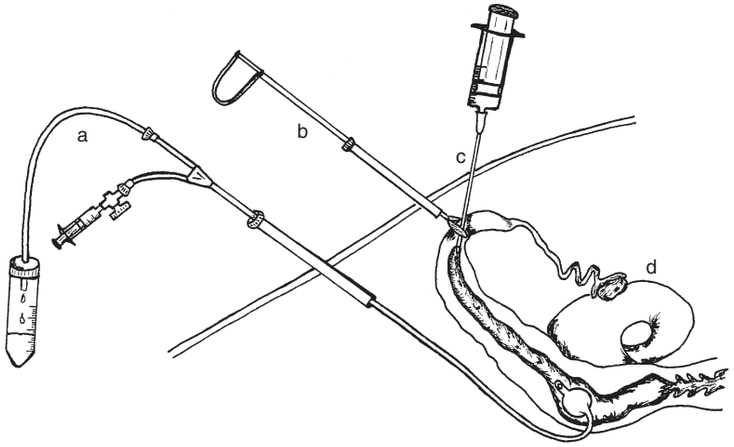

Fig. 83-1 Laparoscopic embryo collection in the goat. a, Ballooned Foley catheter and collection tube; b, laparoscopic atraumatic grasping forceps; c, intravenous catheter (18 gauge, $15^1/_2$ inches long); d, uterine horns.

cradle, a second dose of anesthetic is frequently needed within 30 minutes. In these cases, doses of 0.5 to 1 mg/kg are administered IV as needed.

The abdominal area cranial to the udder is shaved and aseptically prepared for laparotomy or laparoscopy. For the nonsurgical procedures, the perineal area is also aseptically prepared.

Laparoscopic and nonsurgical equipment can be soaked in disinfectant solutions. These solutions are spermicidal and embryotoxic, so instruments should be rinsed thoroughly with sterile water and shaken or otherwise dried prior to their use.

Embryo Collection

The first successful embryo collection and transfer in goats was reported by Warwick and associates.[10] Although their interest was in creating hybrids between goats and sheep, they reported the birth of a kid to a doe following surgical collection of a four-cell goat embryo and replacement of the embryo in the donor's reproductive tract. It was not until 30 years later that further reports on embryo transfer in goats appeared.[11]

In most instances, surgical procedures have been used for the recovery of embryos from goats. However, alternative techniques for collecting embryos from goats, such as laparoscopic and nonsurgical or transcervical embryo collection techniques, have been developed. The embryo collection technique to be used depends on the day that the embryos are to be collected. Embryos can be collected as early as day 1 after breeding, or as late as day 7 or 8 after breeding.[6,12–15]

Early luteal regression (ELR) has been reported in synchronized and superovulated goats.[16,17] Early luteal regression is characterized by small, white-pink corpora lutea and may occur in 10 to 32% of treated animals by the time of embryo collection (days 5–7 after breeding) or transfer. Embryo recovery rates from donor does with ELR are poor; there are observations that ELR can be reduced by administering a luteolysin inhibitor during the period between breeding and embryo collection. Also, progestins or progesterone administered during this period may help reduce the effect of ELR by keeping progesterone levels high during the early stages of embryo development (days 0–7). Another way to avoid embryo loss associated with ELR is to collect embryos on day 3 after breeding, before luteal regression occurs and embryos are expelled from the reproductive tract. Embryos are located in the oviducts at this stage; therefore, an oviductal flush is required.

Surgical Embryo Collection

Although there has been some progress in developing alternative methods for collecting embryos from small ruminants, goat embryos are most commonly harvested by surgical means.[14] This ensures high recovery rates, which can reach 100%. Animals are positioned in dorsal recumbency position with an inclination of 20 to 30 degrees with the head down. Three procedures involving laparotomy have been described for the collection of embryos, all of which involve exteriorization of the uterus through a midventral incision with or without examination of the ovaries to determine the response to superovulation treatment. This procedure should be carried out by skilled surgeons with attention to the principles of surgery; otherwise adhesions of the reproductive tract will develop.

In the first procedure, embryo flushing medium is injected into the uterine horn and expressed out through a fluted plastic catheter inserted into the oviduct through the fimbriated end. This technique can be used for collecting oviductal-stage embryos.[14] In the second procedure, the oviductal contents are flushed from the fimbria into the uterus and out of the uterus through a Foley catheter (size 8 Fr) inserted in the uterine horn near the bifurcation.[18] This technique can be used for collecting embryos at any stage. In the third procedure, the uterine horn is flushed from the tip near the uterotubal junction toward a Foley catheter (8 Fr) inserted in the uterine horn near the bifurcation.[12,19,20] This technique results in fewer adhesions because it eliminates the need to manipulate the oviduct, fimbria, and ovaries. However, the technique is limited to use 4 or more days after estrus, at which time the embryos will have reached the uterus.[21] Embryo collection rates using the surgical approach in goats average 85%.[12,20]

Although anecdotal reports of numerous repeated surgical collections from individual goats exist, several other reports have shown detrimental effects of repeated surgical embryo collections. These effects may be noticed as soon as the second embryo collection is performed in the form of adhesions to the reproductive tract if inadequate surgical procedures are followed during embryo collection.[22] This is a potential limiting factor for the use of embryo transfer for genetic improvement. Therefore, it is important that the surgeon minimize handling the reproductive tract, and once the uterus or part of the uterus is exteriorized, it should be continuously moistened using a soft mist of saline solution and antibiotics.

Laparoscopic Embryo Collection

Laparoscopy has been a useful tool in reproduction for small ruminants. Several researchers have applied this technique to investigate the role of ovarian function in the control of the reproductive cycle,[23,24] to determine the time of ovulation,[25,26] to perform artificial insemination,[27] and to collect and transfer embryos.[12,28,29] It has been reported that in goats the number of ova and embryos collected by laparoscopy compared with the number of corpora lutea (CL) (recovery rate) is approximately 15% lower than through surgery.[12] On the other hand, laparoscopic embryo collection can be repeated successfully on the same goat with few complications. It eliminates the need for exteriorization and manipulation of the reproductive tract and for suturing of the body wall. This prevents formation of postoperative adhesions. However, as mentioned earlier, the same surgical care should be emphasized during these procedures.

As an early alternative to laparotomy for the collection of uterine-stage embryos from sheep, a laparoscope was used to visualize the ovarian response and to exteriorize the tip of one uterine horn at a time through a small incision.[30] Flushing medium was introduced into the tips of the horns through an intravenous catheter and was collected through a glass vaginal speculum placed against the external os of the cervix into a glass recovery dish. This procedure yielded a 46% embryo recovery rate.

Very little research has been reported on the use of laparoscopic embryo collection and transfer in goats.[12,28,29] However, a great deal of laparoscopic embryo transfer research has been conducted in sheep[31] and these techniques are applicable in goats.[32] The donor animal is positioned in the laparoscopic cradle, and pneumoperitoneum is created. The primary trocar and cannula are inserted into the peritoneal cavity approximately 10 cm cranial to the udder and 2 to 3 cm to the left of the midline. Care should be taken to avoid major vessels under the skin and internal organs. The secondary trocar and cannula (5 mm diameter) are inserted to the right of the midline. The laparoscope (5–7 mm diameter) is inserted through the primary cannula and the uterus is located just cranial to the bladder. In some cases, the omentum or visceral fat may obscure the uterus. These can be pushed cranially with a manipulating rod placed through the secondary cannula. Once the uterus is located and the ovulation points counted and evaluated, a third cannula (5 mm in diameter) is inserted on the midline 2 cm cranial to the others. Through this third cannula, a long blunted paravertebral needle (12 gauge, 25 cm long) is used to make a puncture wound in the uterine wall cranial to the bifurcation of the uterus, which is secured through the second cannula with atraumatic laparoscopic forceps. This allows the insertion of a balloon Foley catheter with stylet through the small puncture into the uterine lumen. The balloon on the catheter is inflated and the stylet removed. The uterus is secured by the inflated balloon.

If a three-way catheter is used, atraumatic grasping forceps must be used to block the uterotubal junction to prevent flow of the medium and embryos toward the oviduct. The flushing medium is introduced through the catheter and into the uterine horn by means of an inner extendable tube that reaches the uterotubal junction. Return of the flushing medium is initiated by pressure and gravity. Each horn is flushed with 30 ml of medium, and 79% embryo recovery rates have been achieved.[12]

If a two-way catheter is used (Fig. 83-2), the tip of the uterine horn is secured with atraumatic forceps and cannulated using an intravenous catheter (18 gauge, $5^1/_2$ inches long). After the stylet is removed, a collection medium (60 ml) is introduced through the intravenous catheter and collected through the Foley catheter. The procedure is repeated for the other horn, the instruments

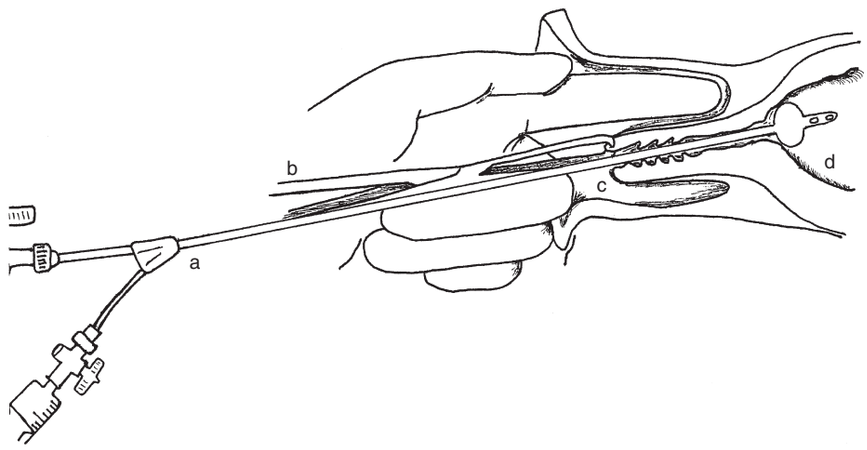

Fig. 83-2 Nonsurgical embryo collection in the goat. a, Flushing catheter; b, Allis tissue forceps; c, vagina; d, uterine body. (From Kraemer DC: *Theriogenology* 1989;31:141.)

are removed, and the abdominal incisions are closed. With this technique collection rates range from 50% to 80%.[29,31]

A postoperative problem could be encountered when a large Foley catheter (>12 Fr) is used. The wound in the uterine wall is usually not sutured; consequently, the endometrium occasionally grows out through the puncture wound to the serosal surface (personal observations). This could interfere with the female's reproductive health. In general, this technique requires considerable practice and skill and has not become the method favored by most practitioners.

Nonsurgical (Transcervical) Embryo Collection

Nonsurgical (transcervical) embryo collection techniques avoid the formation of postsurgical adhesions and maintain the value of genetically superior donors following multiple embryo collections. Moore stated that nonsurgical embryo collection procedures, as used in the cow, would appear difficult owing to the narrow and tortuous nature of the cervical canal of ewes and does.[33]

Nevertheless, reports of successful nonsurgical embryo collection in goats have been published.[34–37] These groups obtained embryos at the blastocyst stage by priming the cervix with a combination of prostaglandins E_2 and estradiol,[38] dilating the cervix prior to flushing with a *Laminaria japonica* tent or mechanically using an oral probe designed for use in rats. The tents were inserted into the cervical canal for 6 to 12 hours and held in place by packing of the cranial vagina with gauze. The flushing procedures were performed in standing animals with a variety of catheters such as a modified two-way 24-gauge Foley catheter through which a stainless steel tube (50 cm long, 1.5 mm inner diameter) was inserted. A Sovereign catheter (5 Fr) was inserted through the stainless steel tube to introduce the medium. Other flushing cannulas were based on the model of the two-way catheter that was developed for use in cattle.[35] The cannula was a concentric double tube with a metallic inner tube (Cathelin needle) for inflow of flushing medium and a polyethylene outer tube for outflow. Using this catheter, the practitioner flushed each uterine horn individually by pulling the catheter back into the uterine body prior to reinsertion into the other horn. Some of the problems encountered during these procedures were the incomplete passage of the cannula through the cervical lumen and the puncture of the uterine wall with the flushing device. Embryo recovery rates were 89.5% for 15 females (34/38 CL), but the collection rate among all the subject animals was not very high.

A method for catheterizing the sheep cervix and collecting embryos nonsurgically without the aid of a cervical dilator was developed by Coonrod and associates.[39] This procedure has been adapted for use in goats.[29,40,41] Animals are tranquilized or anesthetized and positioned in dorsal recumbency. The cervix is visualized using a vaginal speculum and a strong light. A section of the external cervical os is grasped with long Allis tissue forceps. The forceps and the cervix are retracted into the vagina. A catheter (e.g., Verres needle, section of urinary catheter size 8 or 10 Fr with stylet, Ott catheter size 8 Fr) is placed into the external os of the cervix as far in as possible. The vaginal speculum is removed and the index finger of a gloved hand is inserted into the vagina alongside the retracted cervix. The finger is used to guide the catheter through the cervix. Once the catheter is in the body of the uterus or in one of the horns, the stylet is removed and the balloon filled (when catheters with balloon are used) (Fig. 83-3). The tubing for inflow and outflow is connected to the catheter by means of a three-way stopcock, and small amounts of medium (5–10 ml) are introduced at each flush to keep the pressure low and minimize fluid leakage. The catheters without balloons are moved slightly in and out to induce return of fluid. Massaging the abdominal area toward the pelvic cavity helps to recover the flushing medium. The return medium is collected in an embryo filter via the outflow tubing. A novel approach for improving the practicability of the nonsurgical method for embryo collection in goats is the administration of a luteolytic dose of $PGF_{2\alpha}$ 8, 16, or 24 hours before flushing. This procedure will decrease the plasma progesterone concentration and luteolysis and will promote uterine contractility in response to infusion of the flushing medium, increasing from 43% to 80% embryo recovery rates.[36,37]

A combination of a laparoscopic/cervical technique can be carried out. The cervix of a multiparous goat is catheterized with a Foley catheter and the balloon is inflated to secure the catheter and prevent flushing medium from leaking between the cervix and the catheter. Medium is injected under laparoscopic visualization into the tip of each uterine horn collecting the medium via the Foley catheter.[32]

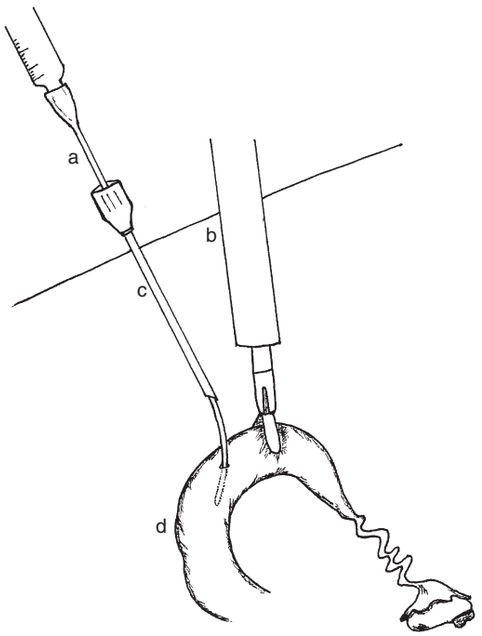

Fig. 83-3 Laparoscopic embryo transfer in the goat. a, Tomcat catheter containing embryos; b, Laparoscopic atraumatic grasping forceps; c, needle (14 gauge), used as a small canula for the Tomcat catheter; d, uterine horn.

Embryo Handling

Flushing medium consists of a solution of phosphate buffered saline to which 10% newborn calf serum (NBCS) and penicillin-streptomycin are added. Embryos are flushed and maintained in this medium throughout the procedure. Following embryo collection, the embryo filter is taken to the laboratory and the contents are poured into a square grid dish while being rinsed thoroughly using a 30-ml all plastic syringe (AIR-TITE, Virginia Beach, VA) containing flushing medium. The embryos are retrieved from the searching dish with an embryo pipette, washed in clean medium, and placed in a sterile two- or four-well covered dish that contains holding medium (phosphate buffered saline + 30% newborn calf serum + antibiotics).

Embryos are carefully examined under a dissecting microscope and an assessment of stage of development and quality is performed. The quality grade is an indication of predicted survivability of the embryo. One of the most common grading systems consists of four categories (grades 1, 2, 3, and degenerate). Grade 1 indicates that the embryo is nearly perfect with more than 98% of the cell mass active and healthy; grade 2 indicates that 70% to 98% of the cell mass is active and healthy and some extruded blastomeres are found; grade 3 indicates a poor quality embryo with less than 70% of the cell mass active and healthy, and several extruded blastomeres are present; degenerate indicates that none of the cell mass appears active and most of the membranes are broken down. Figure 83-4 depicts the different developmental stages of goat embryos.

Embryo Freezing

Embryo freezing or cryopreservation permits the long-term storage of valuable genetics. Embryos can be cryopreserved using conventional freezing[42] or vitrification techniques.[43] Conventional freezing techniques involve the exposure of goat embryos to concentrations of approximately 10% glycerol or ethylene glycol for equilibration. Subsequent exposure to controlled cooling with a computerized embryo freezing unit containing an alcohol bath, and then immersion in liquid nitrogen for storage. Puls-Kleingeld and associates cryopreserved goat embryos by using phosphate buffered saline + 20% heat-inactivated goat serum + 1.4 M glycerol as the freezing medium, and compared the one-step (1.4 M glycerol) and the three-step (0.47, 0.94, and 1.40 M glycerol) equilibration methods.[44] Reports indicated that blastocysts cryopreserved better than morulae, and the one-step technique yielded better results than the three-step procedure (45% versus 19% embryo survival rates, respectively). Vitrification involves (a) exposure of embryos to high concentration of cryoprotectants (20–25% ethylene glycol or glycerol and 20–25% dimethyl sulfoxide or propanediol), (b) loading the embryo in a thinned (pulled to approximately half the original diameter) 0.25-ml straw by capillary action, (c) followed by direct immersion in liquid nitrogen in a vertical position with the thinnest end of the straw first.[42] Vitrification avoids ice crystal formation during the cryopreservation process. The faster cooling rates reduce toxicity of the cryoprotectant and circumvent the chilling sensitivity of embryos. As a result of these advantages, goat embryos have been successfully vitrified[45] with an overall embryo survival rate of 64% (18/28) compared to 42% (10/24) for conventional frozen embryos.[36] Vitrification does not require special equipment; therefore, it may be very well adapted to routine field use with goats.

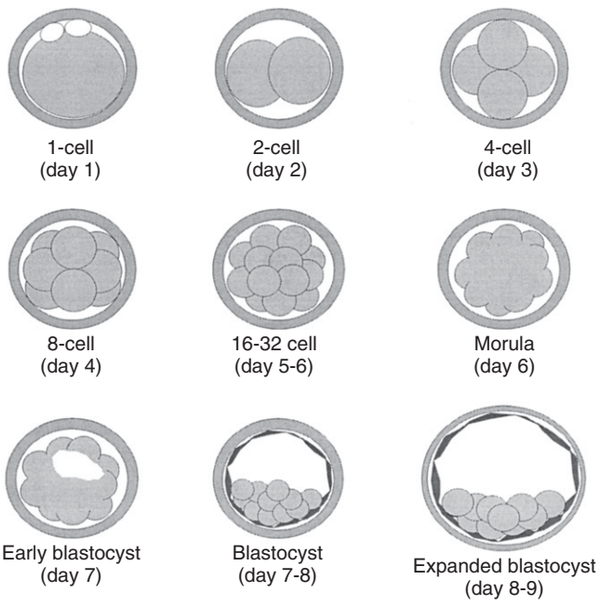

Fig. 83-4 Developmental stages of goat embryos.

Transfer of Embryos

Transfer of embryos can be achieved surgically, laparoscopically, or nonsurgically to recipients that are detected in estrus at the same time as the donor doe, plus or minus 24 hours.[12] Synchrony of donors and recipients is one of the most important factors in the success of an embryo transfer program.

The number of embryos transferred per recipient is correlated with the survival of embryos. Studies indicate that there is a significant improvement when two embryos rather than one or three are transferred. Survival of embryos was also higher when unilateral transfer of both embryos was performed. The possible explanation is that there may be synergy between embryos in influencing each other's survival.[46]

Surgical Embryo Transfer

In most embryo transfer work with goats, surgical procedures similar to those described for sheep have been used. The reproductive tract is approached through midventral laparotomy and exteriorized to allow visual confirmation of a CL prior to transfer of the embryos. Embryos are transferred to the oviducts via the fimbria using a tomcat catheter, Drummond pipette, or Pasteur pipette, if embryos are in the early stages of development (<day 4). Older embryos (>day 4) are transferred to the uterine horns using a Pasteur pipette or tomcat catheter through

a small stab wound made with a rounded needle or with the eye of a suture needle.[12,21]

Laparoscopic Embryo Transfer

Laparoscopic techniques for transferring embryos to a predesignated site within the uterine horn of recipients were developed in sheep.[47,48] The feasibility and efficiency of this technique have been demonstrated in goats.[28,29,49]

Recipient animals are placed in a cradle angled to 40 degrees with the head down. Pneumoperitoneum is produced by introducing CO_2 through a Verres needle inserted transabdominally. Two stab wounds are made in the abdominal wall, each 2 to 3 cm from the midline and approximately 10 cm cranial to the udder. One is for insertion of the laparoscope and the other for insertion of the laparoscopic grasping forceps. Using the grasping forceps, the terminal one half of the uterine horn ipsilateral to the CL is secured. Embryos designated for transfer are drawn into a 3.5-cm Fr tomcat catheter attached to a 1-ml syringe. For cannulation of the uterine horn, a 16-gauge 5.5-cm long Teflon catheter is inserted through the abdominal wall at the site directly ventral to the secured horn. The catheter is gently inserted into the uterine horn and upon penetrating the lumen, the stylet is withdrawn. The catheter is considered intraluminal when it is passed 2 cm and can be manipulated back and forth freely. The Teflon catheter is removed and the tomcat catheter containing the embryos is guided through the cannula and into the lumen of the uterus (Fig. 83-5). After the catheter can be freely maneuvered, the embryos are slowly expelled. The instruments are withdrawn and the incision sites on the abdominal wall sutured.

An alternative technique uses the laparoscope for visualization of the ovulation site on the ovaries, and with the aid of Babcock forceps, a loop of the terminal half of the uterine horn ipsilateral to the CL is grasped and exteriorized through a 2-cm abdominal incision approximately 2 cm from the linea alba (in place of the secondary trocar cannula). Once the section of uterine horn is outside the abdominal wall, a puncture wound is made through the uterine wall with a rounded needle (16 gauge). Through the same perforation, a tomcat catheter containing the embryos is introduced into the uterine lumen approximately 2.5 cm toward the uterine body, and the embryos are deposited.

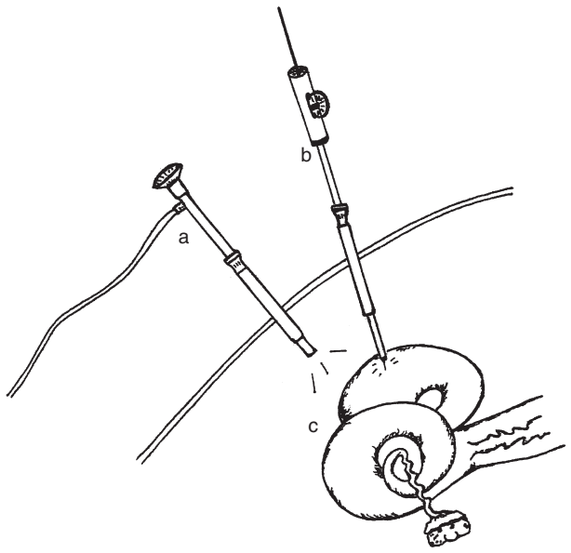

Fig. 83-5 Laparoscopic artificial insemination in the goat. a, Laparoscope; b, laparoscopic inseminating gun; c, uterine horns.

Nonsurgical Embryo Transfer

In small ruminants, manipulation of the genital tract per rectum is not possible. Nevertheless, nonsurgical embryo transfer has been successfully performed in goats by catheterizing the cervix and manipulating the genital tract in a different way.[29,50] The earliest study in goats of a nonsurgical technique to transfer embryos was performed by Otsuki and Soma.[11] With the hypothesis that stimulation of the cervix and consequent release of oxytocin was one of the reasons for failure to obtain pregnancies from nonsurgical embryo transfer, they administered hyoscine-*n*-butylbromide or cocaine hydrochloride prior to transfer in an effort to block parasympathetic nerve transmission from cervical stimulation. The instrument used to transfer the embryos consisted of metal tubes, a polyethylene tube, a 1-ml syringe, cervical forceps, and a vaginal speculum. In the control group (no treatment, $n = 11$), zero pregnancies were obtained; in the group treated with 1% to 2% cocaine hydrochloride as a local anesthetic ($n = 5$), zero pregnancies were obtained; and in the group treated with a subcutaneous injection of 0.02 to 0.03 mg of hyoscine-*n*-butylbromide ($n = 7$), ova implantation succeeded in one case and a female kid was born. In five cases, prolongation of the estrous cycle to 29 to 39 days suggested temporary implantation of the transferred ova.

Another study in which no nerve block was attempted resulted in successful nonsurgical embryo transfer in Taiwan goats using a 16-gauge, 7.5-cm catheter and cervical manipulation.[50] The cervix was catheterized by introduction of the left index finger of a gloved hand into the vagina to manipulate the ventral surface of the cervix and guide the catheter into the uterus. This technique resulted in five of eight females pregnant, and produced a total of six fetuses. The authors believed that the short length and rigidity of the catheter plus manipulation of the cervix made the procedure very easy and contributed to its usefulness. Pregnancy rates of approximately 40% after nonsurgical embryo transfer have been reported in which the cervix of recipient goats was catheterized with an intravenous catheter (14 gauge, 14 cm long; Abbott Hospitals, Inc., North Chicago, IL), which has a modified rounded smooth stylet to stiffen the catheter.[29,51] During cervical catheterization, embryos are loaded in an intramedic tubing (17 cm long, 0.76 mm inner diameter; 1.22 mm outer diameter) connected to an 18-gauge needle attached to a 1-ml syringe. Once the cervix is catheterized, the intramedic tube containing the embryos is passed through the catheter and into the uterus. The practitioner expresses the embryos into the uterus by gently pushing the plunger of the syringe.[29]

FOLLICLE ASPIRATION

In vitro produced embryos can originate from oocytes collected from postmortem ovaries or from live donors by follicle aspiration. Compared with oocytes from postmortem ovaries, oocytes aspirated from live animals are of known health status, which could be of vital importance for the production of transgenic animals.[52] Bovine oocytes are commonly collected by ultrasound-guided transvaginal "ovum pick up" (OPU). In small ruminants, oocytes from live animals are obtained by laparotomy[53] or by laparoscopy,[54] but there are reports in goats in which ultrasound-guided transvaginal oocyte aspiration were performed. In this experiment, goats were subjected to either a laparoscopic aspiration or a transvaginal ultrasound-guided aspiration, and results demonstrate a similar percentage of oocyte recoveries (71 versus 68%, respectively).[55,56] Follicle aspiration by laparoscopy is simple and allows the repeated production of IVF (in vitro fertilization) or NT (nuclear transfer) embryos from live donors of particular value with or without the need of superovulation. However, repeated laparoscopic aspirations may lead to adhesions in the oocyte donor does and the techniques demand great skill.[55] Oocyte retrieval after laparoscopic aspiration from unstimulated goats provides 4 to 6 good oocytes per female.[57] Gonadotropin treatments of a multi-injection (133 mg NIH-FSH of Folltropin-V) or a single injection regimen (80 NIH-FSH of Folltropin-V and 300 IU eCG) for stimulation of oocyte donors have also been used and similar numbers of oocytes have been obtained (12.7 ± 5.6 versus 10.0 ± 5.7 oocytes, respectively).[52]

For laparoscopic follicle aspirations, animals are placed in a standard laparoscopic position and under general anesthesia. Pneumoperitoneum is produced by introducing CO_2 through a Verres needle inserted transabdominally. Two stab wounds are made in the abdominal wall, each 2 to 3 cm from the midline and approximately 10 cm cranial to the udder, one for the insertion of the laparoscope and the other for the atraumatic forceps. Under laparoscopic observation, a short beveled 20-gauge 2-inch long paravertebral needle is passed through the abdominal wall to aspirate the follicles on the surface of the ovaries while the ovary is secured at the mesovarium by the atraumatic forceps. The needle is connected to a collection tube containing Tyrodes lactate HEPES medium, 100 µg penicillin, 100 mg streptomycin, and 2500 units of heparin for every 100 ml of medium, and via Teflon tubing, to a vacuum pump (25 to 50 mm Hg).

The growing interest in the production of embryos from prepubertal animals (3 to 5 months of age) has encouraged numerous researchers to investigate the developmental competence of oocytes from prepubertal females.[58–60] Results have shown that oocytes from prepubertal goats are capable of undergoing IVM, IVF, and developing to the blastocyst stage in culture at rates similar to those of oocytes from adult goat ovaries.[59,61] Baldessarre and associates reported acceptable pregnancy rates from oocytes aspirated from prepubertal goats following IVM-IVF.[52] This offers a useful tool for fundamental research and a way to shorten the generation interval.[62]

IN VITRO MATURATION AND FERTILIZATION

The first goat kid produced by in vitro fertilization of ovulated matured oocytes was produced by Hanada.[63] In vitro maturation (IVM) and fertilization (IVF) in goats have resulted in several kids. In vitro embryo production requires precise methodologies to successfully produce transferable embryos and subsequent pregnancies. The process includes oocyte maturation, sperm preparation, fertilization, and culture. Each of these must be developed individually and then combined into an integrated program. IVF offers the advantage of producing embryos from animals when production of embryos in vivo is difficult or impossible. In addition, efficient IVM and IVF procedures are important for the development of other biotechnologies such as embryo splitting, sexing, nuclear transfer, and gene transfer. Oocytes for IVM and IVF may be of postmortem origin or from follicle aspiration from the ovaries of live animals. Ovaries collected post mortem (untreated females) provide an abundant source of oocytes at low cost. Ovaries can be minced or follicles can be aspirated in the laboratory to obtain the oocytes. Cumulus-surrounded oocytes and homogeneous ooplasm recovered from 2- to 5-mm follicles are washed in TALP-HEPES (Sigma) twice and then incubated in M199 (Earle's salts) supplemented with 20% goat serum plus 4 µg/ml FSH and 4 µg/ml LH for 24 hours in a 5% CO_2 atmosphere at 39° C. Maturation rates of approximately 90% have been obtained in goats.[66]

In vitro fertilization is commonly performed with frozen-thawed spermatozoa. The frozen-thawed sample is subjected to one of two methods for separating live-dead cells: (a) Percoll density-gradient centrifugation and (b) swim-up in sperm-Tyrode's lactate at 37° C. The Percoll method yields a greater number of motile sperm after a 25-minute process when compared to the swim-up method, which is a 2-hour process. As the motile sperm cells swim up in the medium, the supernatants are collected and motile spermatozoa are recovered.

Regardless of the sperm separation method, the concentration is measured with a hemocytometer and, if needed, sperm are diluted to the final concentration of 1×10^6 cells/ml.[66] One of the most important components of the procedure is thought to be capacitation of the spermatozoa.[67] Heparin is commonly added to the fertilization medium to capacitate the sperm cells, which leads to acrosome reaction and will enhance fertilization rates. However, the quality of the embryos produced when heparin is added to the IVF medium is questionable. Embryo survival rates demonstrate higher percentage of viable kids when capacitation is induced with heat-inactivated estrous serum than with heparin (61% versus 25%).[66,68] An average of 70% normally fertilized goat oocytes can be routinely obtained in vitro, indicated by the presence of both the male and female pronuclei or by cleaved ova within 48 hours of culture.[69] The incidence of polyspermy in goat oocytes has been reported to be relatively high following IVM-IVF, affecting nearly 20% of the fertilized oocytes.[66]

Progress in the culture of IVF goat embryos has been made: domestic chicken eggs have been used to culture

in vivo collected goat embryos to the blastocyst stage,[70] and more recently, coculture systems with oviduct epithelial cells have been used successfully to produce blastocysts and live offspring following embryo transfer.[59,71,72] Several researchers have successfully used synthetic oviductal fluid to culture goat IVM-IVF embryos and obtained live offspring (61% resulted in live kids) after transfer of blastocysts.[73,74]

MICROMANIPULATION OF OVA AND EMBRYOS

Intracytoplasmic Sperm Injection

The process of assisted fertilization by intracytoplasmic sperm injection (ICSI) has been used in human fertilization clinics for treatment of male infertility. In farm animals, ICSI has been used to study methods to overcome problems encountered during IVF, such as sperm capacitation and poor or nonmotile sperm. Studies indicate that fertilization by sperm injection varies between species; therefore, further studies are required to establish protocols for sperm treatment and oocyte activation in the goat. Following ICSI in goats using IVM oocytes, embryos have been produced that successfully developed to the blastocyst stage.[75]

Embryo Splitting

Early attempts to produce identical animals were based on embryo splitting. Embryo splitting produces monozygotic twin individuals, which offer various possibilities for experimentation and production traits studies. A few reports on the splitting and transfer of goat embryos demonstrate the feasibility of this technology.[76,77] Briefly, while viewing under a microscope (75×), a glass suction micropipette is used to hold the embryo, and the zona pellucida (ZP) is opened with a finely drawn glass needle. The embryo is surgically dissected with a glass knife into halves or embryo parts. One of the halves is left in the original ZP and the other half is placed into an empty ZP. "Half" embryos, or demi-embryos, can be cultured, transferred immediately to synchronized recipients, or frozen.[78,79] Pregnancy rates of 36% have been obtained after transferring two goat fresh demi-embryos each to 11 recipients.[76] Goat embryos seem to be very sensitive to micromanipulation compared to other livestock embryos, and to increase demi-embryo survival rates, micromanipulation techniques need to be improved. Cells in the morula stage are loosely associated; thus, manipulations cause the disintegration of most embryos. Tsonuda and associates demonstrated that hatched blastocysts were most suitable for goat embryo bisection.[77] Demi-embryos have been frozen inside a zona pellucida or without a zona pellucida and results following embryo transfer demonstrate a lower kidding rate from demi-embryos frozen without a zona pellucida.[79]

Nuclear Transfer (Cloning)

This technique is applied for the production of genetically identical animals. Early work in nuclear transfer involved the use of totipotent blastomeres and inner cell mass cells from early embryos to create identical animals.[80,81] In the goat, normal identical kids have been produced by transferring blastomeres from a 4- to 32-cell embryo into an enucleated mature oocyte.[82] It was not until Dolly's birth[83] that adult somatic cells were successfully used by different laboratories to produce cloned offspring.[84–87] The first cloned goats by somatic cell nuclear transfer were produced by Baguishi in 1999.[85] Nuclear transfer involves the production of enucleated oocytes, called a cytoplast, that serves as sufficient medium to reprogram and express the genetic heritage of the donor nucleus. Oocytes in metaphase II from in vivo or in vitro sources are used for this purpose. Enucleation involves the removal of the metaphase spindle with its associated maternal chromosomes from the mature unfertilized oocyte. This procedure is usually performed with the aid of a DNA-specific fluorochrome, which allows visualization of the chromatin and confirmation of successful removal under ultraviolet light.[88] Commonly an intact donor cell is injected into the perivitelline space of the enucleated oocyte and their membranes electrofused. An alternate technique involves the direct injection of the isolated nucleus into the enucleated cytoplasm.[89,90] In goats, both skin fibroblasts and cumulus-granulosa cells have been used successfully as donor cells.[91,92] The transferred nucleus goes through various changes characterized by remodeling of the nucleus, which presumably results in reprogramming of the transferred nucleus so that it behaves developmentally as if it were a one-cell zygote.[93] Although embryonic development can be activated by the electrofusion step in some species, it is common to chemically activate the oocytes with a combination of an ionophore and a protein kinase inhibitor. The successfully fused and activated cells are placed in culture or are transferred into a synchronized recipient immediately.

In the context of animal husbandry, reproductive cloning can be applied to rapidly upgrade the quality of a flock by cloning an animal noted for its exceptional production traits; it can also be applied to reproduce an animal that cannot reproduce naturally nor by assisted reproduction (i.e., castrated, ovariohysterectomized) or deceased animals. To apply this technology, a skin biopsy is taken from the animal to be cloned, the cells are grown in tissue culture flasks in an incubator and when the cells reproduce in vitro and form a monolayer, they are usually trypsinized and passaged several times to produce around 10 million cells, which are then cryobanked using cryoprotectants such as dimethyl sulfoxide. It is of great importance that tissue banking is performed from exceptional animals when they are alive. In case of an unexpected death, the carcass should be cooled to 4°C as soon as possible and tissue samples collected and cultured as discussed previously.

Embryo Sexing

Methods to determine the sex of embryos before transfer have traditionally focused on cytologic techniques[94] or the use of antibodies to male-specific antigens such as the H-Y antigen.[95] Now molecular procedures such as poly-

merase chain reaction (PCR) also offer opportunities for embryo sexing.[96] Removal of a few cells from preimplantation embryo is necessary. Amplification of single copy genes (sex-specific sequences) in small samples such as embryonic cells is performed by PCR, with subsequent visualization of these sequences on agarose or polyacrylamide gels. This technique has been applied in goats to amplify these sequences from male and female genomic DNA to identify the sex.[97]

Gene Transfer

The dramatic advances in molecular biology and the development of new technologies like gene transfer have provided a potential tool for genetic improvement of domestic animals. The process of gene transfer adds specific genes to existing animal breeds to enhance production characteristics or to produce pharmaceutical products from milk of goats expressing the transgene in their mammary gland. One of the methods successfully applied in goat studies for incorporation of specific genes into embryos is accomplished by microinjection of the recombinant DNA into the pronuclei of recently fertilized eggs, followed by embryo transfer into synchronized recipients.[98] Between 500 and 1000 copies of the genes are injected into the male pronucleus. After the injection, embryos are either cultured or transferred directly to the recipient goat. The rates of incorporation of the foreign DNA and the survivability of injected goat embryos is low, with only 1% of injected embryos resulting in transgenic goats.[98,99] A more predictable method is by transfection of a cell line followed by nuclear transfer. The donor cells that produced the first cloned goats by somatic cell NT were derived from a transgenic fetus.[85,87]

Dairy goats have been used to produce a commercial prototype for large-scale manufacture of high market-volume proteins in their transgenic mammary gland. To achieve protein expression, a human gene controlled by milk protein promoter sequences is inserted into the surrogate embryos (goat) and when the resultant transgenic animals begin to lactate, they secrete the human protein in their milk. The protein is then purified from milk. Several specific reasons were considered in choosing goats: (1) They produce large volumes of milk; (2) they have short gestation and developmental periods; and (3) the biochemical features of goat milk have been extensively characterized. One of the therapeutic proteins expressed by transgenic goats in their milk has been a longer-acting tissue plasminogen activator protein, which has a tremendous pharmaceutical value to cardiac patients.[99] Incorporation, expression, and transmission of the foreign gene in domestic animals occurs at low frequencies; therefore, extensive research continues.

Marker-Assisted Selection

Increasingly today DNA technology has been advancing to identify genetically important traits. Utilizing genetic markers, some traits like disease resistance or growth and carcass performance, can be screened in individual animals and flocks to identify and perpetuate those animals with economically beneficial characteristics. Also animals carrying an undesirable trait can be screened and eliminated for a more profitable goat industry.[100] Marker-assisted selection incorporated with AI, ET, and cloning technologies are expected to accelerate the production of animals that exhibit desirable traits.

The techniques for isolating DNA can be used for screening embryos, semen, blood, and tissue banked cell lines. Therefore, tissue banking from individual animals will allow for the possibility to screen for genetic markers and evaluate past, present, and future production performances.

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CHAPTER 84

Reproductive Physiology of the Ram

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An understanding of basic ram reproductive physiology forms the foundation to the designing of practical approaches to management of a breeding program and improved reproductive efficiency. In addition, this knowledge has the potential to improve our ability to advance flock genetics through indirect selection for reproductive merit.

ORGANIZATION OF THE REPRODUCTIVE SYSTEM

The cryptorchid condition can be used to illustrate some important concepts about the developmental organization of primary and secondary sex organs and reproductive development in the ram. Histologically, seminiferous tubules of cryptorchid testes are juvenile in appearance and devoid of germ cells[1]; however, despite the fact that these rams are sterile, few differences in other male traits occur. Growth rate, male-type body and carcass characteristics, and the accessory sex glands are little affected. A similar situation is seen in rams with short scrotums who, although usually sterile, nonetheless reach normal physical maturity.

The cryptorchid and short scrotum conditions can be used to gain information about male reproductive development. First, reproductive competence and fertility in rams depend on migration of the testes to the scrotum at birth. The scrotum is where the testes reach full maturation and where spermatozoa acquire fertilizing potential. In hot humid areas such as the southwestern desert in the United States, the scrotum affords some protection against the deleterious effects of heat, although even with this efficient cooling mechanism, temperature-induced sterility is often a problem. Although scrotal descent is essential for fertile sperm production, it is not needed for all endocrine functions of the testes. Testes formed during in utero development are fully capable of secreting androgens.[2] It is this organizational effect of androgen, occurring 30 to 40 days after conception,[3] that directs differentiation of the reproductive tract toward the male system.

The hormone inhibin, which is produced by Sertoli cells within the seminiferous tubules, is lost together with the germinal epithelium in the cryptorchid state. However, the androgen-producing cells (Leydig cells) continue to have the capacity to secrete. Androgen secretion throughout the juvenile period of the ram lamb stimulates growth and maturation in cryptorchid or normal rams.

During gestation, sexual organization of neural tissue occurs under the direction of the testes. Among the many neural systems undergoing profound changes, two are critical. The first involves development of the hypothalamus, which produces and secretes gonadotropin-releasing hormone (GnRH). The second is the development of brain regions involved with sexual behavior.

Gonadotropin-releasing hormone plays a pivotal role in male reproduction, directing the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland, and ultimately affecting testicular function. Although marked gender differences are observed in the secretion of LH, and presumably of GnRH,[4] no differences in neuroanatomy (number or appearance of GnRH neurons) have been found between male and female sheep.[5] This suggests that androgens in utero alter the secretory capacity of the GnRH neuronal network and thereby establish distinct male and female patterns of release.

Fetal testicular androgen secreted during early development organizes hypothalamic and extrahypothalamic neurons involved with the expression of sexual behavior in the adult ram. Masculinization, or the ability to express male sexual behavior, is established in utero between days 20 to 80 of gestation.[3] Androgens given to pregnant ewes by mid-gestation masculinize female offspring. As adults, such ewes not only fail to show typical estrous behavior and ovulate, but display courtship and mounting behavior typical of rams.

ACTIVATION OF THE REPRODUCTIVE SYSTEM

The appearance of spermatozoa and the onset of mating behavior that herald the initiation of puberty in rams usually occur at 5 to 6 months of age, depending somewhat on breed and season of birth.[6] Spring-born rams from temperate climates show a gradual increase in testicular size that parallels changes in growth rate with a more rapid phase of testicular growth occurring in the fall. Changes in GnRH-induced LH secretion drive the final maturation of the testes, including stimulation of testosterone secretion. Onset of mating activity is synchronized by changes in steroids. The rise in LH secretion that occurs during the peripubertal period is likely caused by (1) a steroid-independent drive of hypothalamic GnRH secretion and (2) a change in feedback inhibition of testosterone on the GnRH/LH axis.[7]

The importance of GnRH and LH to drive events that lead to puberty is further shown by differences in age at which inadequately nourished rams reach sexual maturity,[8] because low planes of nutrition are associated with inadequate increments in LH release.

Seasonality

Testicular size, semen quality, mating behavior, and wool growth change during the year in rams in temperate climates. Photoperiod is the primary cue that regulates this annual rhythm. Two mechanisms have been identified that explain these cycles. The first involves an inherent, steroid-independent mechanism of GnRH secretion. During transitions from long to short days, the hypothalamus becomes more active in the secretion of GnRH, stimulating secretion of LH and FSH from the pituitary. Under the influence of increasing gonadotropin levels, a cascade of events occurs, including growth of the testes, stimulation of sperm production, and increased mating behavior. The regulation of GnRH and subsequent gonadotropin secretion depends on the negative feedback effects of androgen (testosterone) from the testes. During transition periods into and out of breeding seasons, testosterone varies in its effectiveness as an inhibitory hormone to GnRH and gonadotropin secretion. Melatonin is responsible for the seasonal change in testosterone feedback sensitivity and, ultimately, gonadotropin stimulation of the testes. It is secreted from the pineal gland during the dark portion of the photoperiodic cycle. During the shorter days of the fall season, blood melatonin concentration is elevated for a longer portion of the day. The melatonin and GnRH axis has evolved as an effective system to translate the environmental effect of season. This has ensured that under natural conditions mating occurs in the fall, and lambs are born at a favorable time of year.

Sexual Behavior

The activation of mating behavior in rams occurs at puberty, thereafter occurring seasonally in the mature adult. Ram lambs castrated at birth rarely show onset of courtship behaviors because of the absence of testosterone. Replacement of testosterone in a castrated male will elicit onset of sexual behavior and, at the appropriate threshold dose, copulation. Proficiency in courtship and mating has learned components that can be observed when lambs are weaned. Ram lambs frequently mount other males when they are put in all-male groups (an important consideration for disease transmission). Rams that are reared with females can achieve an earlier onset of mating activity. The frequency of various courtship behaviors varies by season of the year, occurring less frequently and less intensely in periods that are typical of anestrus in ewes. The seasonal changes that occur in mating behavior should not be confused with situations in which rams fail to perform sexually. Sexual performance is highly variable among rams, irrespective of season. Both sexual inactivity and homosexual behavior have been described, and approach an incidence of 10% to 20% in populations of rams.[9] In a series of studies designed to elucidate physiologic correlates to these abnormal behaviors, differences in gonadotropin secretion and receptors in the brain for estradiol were found.[10–12]

BEHAVIOR

Rams have characteristic courtship patterns that precede mating. Typically a ram approaches a ewe in a low stretch position with the head angled to the side. Often the ram contacts the flank of the female, kicks out a foreleg, and sniffs the vulva. After contact by the ram, the ewe often urinates. The ram sniffs both the vulva and urine, and the flehmen response usually occurs in which the ram arches the head upwards and with an open jaw draws odors rapidly over the nasal passage and turbinates. The flehmen response is believed to facilitate the detection of estrus. Some rams will mount several times before ejaculating, and others may service a ewe during the first mount. Furthermore, sexually inexperienced rams or ram lambs often have orientation problems with respect to mounting behavior. Young rams are typically subordinate to more experienced rams and the dominance order can affect the efficiency of multiple-sire mating. At least three rams per multi-sire pen are recommended to lessen dominance effects. Within a group of ewes, rams also show some preference for certain females while ignoring others in estrus and this accounts for some differences in the distribution of lambing.

SPERMATOGENESIS

The spermatogenic cycle takes approximately 6 weeks in mature rams. Stem cells are the precursor stage, and not all stem cells start the cycle at the same time; rather, cell division of groups of cohort cells occurs every few days, thus ensuring a continuous flow in sperm production. Although sperm appear normal when they arrive within the seminiferous tubules after commencing their journey 6 weeks earlier, they are not fully mature. During their passage from the seminiferous tubule to the epididymis, which takes about 2 weeks, changes occur that render the sperm fully fertile. Heat stress or other environmental effects that interrupt the flow of cells can cause a ram to be temporarily sterile. It can take up to 6 weeks, or one full cycle, for a ram to recover. Sperm production is related to testis weight and size with rams producing large numbers of sperm per gram of testicular tissue.

APPLICATION OF PHYSIOLOGIC PRINCIPLES

The previous sections have outlined some physiologic principles involved with reproduction in rams. The following sections raise some questions about past application of this knowledge but also present some potential new applications in the future.

Testes Size

The testes are readily palpable endocrine organs that reflect changes in season and reproductive physiology.

Land[13] first proposed using testes size as a selection criteria. However, there are many conflicting studies, which make any recommendations about indirect selection for testis size and flock reproductive potential questionable.[14] Some studies show that determining ram-to-ewe ratios by volume of testes can extend "ram power" for the short term.[15] Yet under most conditions, testes size is not a limiting factor,[16,17] nor is a difference in semen quality correlated with size of the testes.[18] Perhaps rather than a general recommendation of "bigger is always better," testes size of fertile rams should be taken only as a primary factor for specialized breeding conditions, such as low ram-to-ewe ratios, large numbers of synchronized ewes, transitional period to anestrus when semen quality is limiting, and use of ram lambs for breeding. Excellent semen quality, no palpable lesions of the testes or epididymides, a negative test for brucellosis, and outstanding sexual performance are more appropriate criteria to be considered in ram selection.

Gonadotropin-Releasing Hormone for Selection

The response of rams to an injection of GnRH has been evaluated as a potential means of assessing degrees of reproductive competence. Because LH secretion in rams is episodic and random, the use of GnRH decreases variability when LH is measured. Furthermore, both ewes and rams share this system, making the measurements in rams a potentially valuable indirect selection criterion that can be correlated with ewe reproductive efficiency. In an experiment using data from eight generations of selection for GnRH response, significant divergence in LH secretion was observed.[19,20] Interestingly, a correlated response in ewes showed a tendency for earlier onset of sexual maturity and a higher ovulation rate at the beginning of the breeding season. Replication of these data in other breeds and environments is needed, but application of this technique may some day lead to more rapid ways of selecting flock replacements.

Serving Capacity

Serving capacity is a measure of mating ability. Serving capacity tests measure the number of ejaculations or services during a period of time. This test can be predictive of success in pen-mating.[21] The relationship between ram contacts in field mating conditions with overall lamb production[22] suggests that serving capacity should be given credence as part of a breeding soundness examination. Improvements in flock reproductive performance using serving capacity tests to screen rams occur through more lambs per ewe joined, a decrease in the length of the lambing season, and a more uniform lamb crop. Serving capacity as a measure of ram performance goes beyond these short-term gains in flock breeding. Measures of sexual performance suggest a relationship to long-term genetic gain. Libido and other aspects of male mating performance have been shown to be heritable in both beef and poultry. Although selection studies with rams have not yet been accomplished, there is indirect evidence of a genetic relationship. Ewes selected for improved performance in pounds of lamb weaned produce rams with higher serving capacity scores.[23] Interestingly, rams born co-twin to a male sibling also tend to have higher serving capacity scores.[24] The application of serving capacity tests for long-term genetic progress depends on development of paradigms for identifying superior rams. Physiologic indicators of ram performance may prove valuable for screening rams, making this aspect of evaluation more practical in the future.[25]

Seasonality

Technology to change the breeding performance of both rams and ewes and reduce seasonal constraints is available. Two principal approaches have been used to manage rams for optimal breeding performance at any time of the year. One is to house rams in barns to control the length of the photoperiod. This technique has been used to elucidate endocrine patterns and improve fertility of rams for out-of-season breeding. Novel light schedules suggest that indoor housing facilities can be made to serve more practical functions.[26] In one such scheme devised in France, rams kept in barns under light control have less dramatic changes in testes size. The light control schedule consists of changing the daily light pattern from 8 hours of light to 16 hours of light per day every 60 days. This more frequent light and dark contrast shows promise for reducing seasonal breeding limitations. An option for extensive operations where lighted barns are not practical is to use an implant of melatonin. These implants alter seasonality in rams and improve lamb production out of season.[27] Availability of implants to producers remains a problem, however.

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CHAPTER 85

Breeding Soundness Evaluation and Surgical Sterilization of the Ram

CLEON V. KIMBERLING and GERILYN A. PARSONS

Breeding soundness evaluation (BSE) is an overall assessment of the ram's capacity for serving and impregnating a number of ewes during a breeding season. An overall physical examination is performed with special emphasis on the reproductive system. The BSE includes anatomic and structural correctness, freedom of disease, body condition, scrotal circumference, and semen quality. The ram accounts for the major genetic changes in a flock. An economic soundness evaluation (ESE) is an assessment of the ram's potential contribution to the profitability of a sheep enterprise. An ESE includes an evaluation of ram management and an assessment of the potential genetic contribution of the ram.

ECONOMIC SOUNDNESS EVALUATION

The ram genotype influences not only the quality but also the quantity of production per ewe exposed. A highly fertile ram can increase lamb crops by impregnating more ewes and by producing more multiple births per ewe.[1] Research has shown that over three generations the ram is responsible for approximately 87.5% of the genetic influence on a flock.[2] Therefore, specific criteria

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Table **85-1**

Heritability Estimates

Trait	Heritability (%)
Reproductive	
Ewe fertility	5
Prolificacy	10
Scrotal circumference	35
Age at puberty	25
Carcass	
Carcass weight	35
Weight of trimmed retail cuts	45
Percent trimmed retail cuts	40
Loin eye area	50
Fleece	
Grease fleece weight	35
Clean fleece weight	25
Percent yield	40
Staple length	55
Fiber diameter	40

should be established for both production and reproductive traits.

Heritability

The estimated heritability of different traits varies greatly. Although many production traits, such as carcass, dairy, and wool characteristics, are highly heritable, most reproductive traits are not (Table 85-1).[3] Selection pressure for traits with low heritabilities must be intense over several generations for progress to be noted. Genetic progress is generally slower when selecting for multiple traits. It is also important to be aware that selection for some traits may have a negative impact on others. For example, many carcass characteristics and wool characteristics are negatively heritable.

Scrotal circumference (SC) is probably the most important genetic trait associated with reproduction efficiency. The heritability of SC is estimated at 35%. SC is correlated with sperm output, age at puberty of the female offspring, ovulation rate, and the number of multiple births produced. In addition, some British researchers have reported a positive correlation between SC and the ratio of muscle to fat in the offspring. Failure to properly select for SC has the potential to reduce reproductive performance and production, thereby reducing the potential profitability of subsequent generations.

Cost of Ram Power

The cost of ram power per lamb produced is an important component of the ESE. The cost of ram power is determined by purchase price and by the number of lambs sired by an individual ram. Table 85-2 gives an example of annual costs per ram, exclusive of purchase price. Based on this example, Table 85-3 shows how the ram-to-ewe ratio and lambing percentage affect the annual cost of a ram per lamb produced.

Table **85-2**

Annual Ram Costs*

Example Purchase Price $350.00	
Fixed Costs	
Depreciation/3y	117.00
Interest on investment at 12%	42.00
Total Fixed Cost	$159.00
Variable Costs	
Feed and maintenance	60.00
Overhead	5.00
BSE	15.00
Death loss/15%	52.50
Total Variable Costs	132.50
Total Costs	291.50
Income from Wool	20.00
Annual Cost per Ram (exclusive of purchase price)	271.50

*These figures are representative of Colorado producers as reported by CSU farm management specialists Rod Sharp and Paul Gutierrez.

Table **85-3**

Estimated Cost of Ram Power per Ewe Exposed and per Lamb Produced

		COST PER LAMB PRODUCED, $	
RAM:EWE Ratio	Cost per EWE Exposed, $	125% Lamb Crop	175% Lamb Crop
1:1	271.50	—	—
1:25	10.87	8.70	6.20
1:50	5.43	4.74	3.10
1:100	2.71	2.17	1.55

Breeding Capacity

The breeding capacity of a ram is determined by sperm production, semen quality, and libido. The breeding capacity of a highly fertile ram is one of the most difficult concepts for many sheep producers to accept. Under most range and semiconfinement conditions, a ram in good physical condition, with high libido, can successfully serve 100 or more ewes during a 17-day breeding cycle. For example, one 5200-ewe western range operation using 1.5 rams per 100 ewes consistently lambs over 90% of the bred ewes during the first 17 days of the lambing period and averages a 140% lamb crop.[4] In this type of an operation, a higher ram-to-ewe ratio cannot be economically justified.

BREEDING SOUNDNESS EXAMINATION

The BSE is one of the most essential components of the overall economic soundness of a sheep operation.

Reproductive efficiency is a key element of economic efficiency.

Brucella ovis

Brucella ovis is the primary cause of lowered fertility in multi-sire breeding systems in the western United States. *B. ovis* is also a major concern in Australia and New Zealand. *B. ovis* infection in a flock can have a devastating effect on the level of production by decreasing the number of ewes bred, decreasing the number of multiple births, and increasing the lambing interval. *B. ovis* ram epididymitis is a contagious venereal disease generally affecting mature rams in multi-sire breeding systems. The disease is transmitted via homosexual activities or via the ewe during the breeding season. The ewe does not become permanently infected, but serves as a mechanical vector for the spread of the disease.

The *B. ovis* organism enters the blood through the mucous membranes and infects the reproductive tract. Clinical signs include enlargement and fibrosis of the epididymis with wasting of testicular tissue. In some cases, infection of the secondary sex organs occurs without involvement of the epididymis. In these instances, no palpable lesions are evident, and diagnosis must be made by microscopic examination of the semen, semen culture, or serology. Table 85-4 shows a comparison between the semen qualities of a *B. ovis*–free and a *B. ovis*–infected ram population.

Antibiotic treatment of *B. ovis* is disappointing, as about half of the cases do not respond. Vaccination interferes with the enzyme-linked immunosorbent assay (ELISA) serologic test, and is strongly discouraged. It is recommended that all rams in a multi-sire flock have an annual ELISA test, preferably during the period of lowest sexual activity. In single-sire flocks, the ram should be tested at purchase and retested 30 days later. All rams that test positive should be immediately culled. With the potential of *B. ovis* being in a latent stage and not showing a positive test in a multi-sire flock, the entire group of rams should also be retested at the end of the breeding season/shearing, once again culling all positive rams.

Table **85-4**

Semen Quality of a *Brucella Ovis*-Free and a *Brucella Ovis*-Infected Ram Flock

	B. Ovis-Free	*B. Ovis*-Infected
Rams, *n*	84	94
B. ovis ELISA positive, *n*	0	72
Grossly palpable epididymal lesions, *n*	0	23†
Motility, %	66	29
Semen parameters, mean		
Normal sperm, %	86.5	55.5
Detached heads, %*	6.9	25.5

*In an infected population, *B. ovis* infection is correlated with seminal leukocytes and detached spermatozoal heads.
†Removed without semen evaluation.
Data from *Ram Breeding Soundness Evaluation Records*, Fort Collins, CO: Department of Clinical Sciences, Colorado State University, 1990.

Libido

Libido refers to the willingness of a ram to breed ewes. A serving capacity test or direct observation of a ram with ewes is an important component of the BSE, but is frequently neglected. It has been estimated that approximately 10% of all rams have no interest in breeding ewes. In addition to not siring lambs, these rams can interfere with other working rams and should be removed from the breeding population. The behavioral evaluation of a ram should be conducted before the evaluation of the reproductive tract and the semen evaluation. Rams with inadequate libido should be culled without incurring further expense.

Physical Examination

Restraint can vary, depending on the number of animals being tested and the number of people available to assist. The most important factor in restraint is that it should allow for a complete examination of both structural and physical characteristics. An adequate examination can be conducted on a standing ram restrained by a halter and pushed against a wall or fence. However, a calf table or sheep cradle makes restraining a ram for proper examination much easier.

Structural soundness refers to the capacity of a ram to remain sound during the breeding season under normal environmental stresses. The feet, legs, and teeth should be examined for structural soundness. *Physical* soundness refers to the health of the ram. The ram should be examined for conditions that may prevent optimal performance, or that can be transmitted to the ewes, such as foot rot, lip and leg ulcerations (ulcerative dermatitis), and pizzle rot. Body condition should also be noted during the physical examination. A body condition score of 2.5 to 3.5 is recommended for rams entering the breeding season. Structural and physical conditions should be noted at the time blood is collected for the *B. ovis* ELISA test, in conjunction with libido testing, and before examination of the reproductive tract and semen evaluation. If there are structural or physical conditions that cannot be corrected, the ram should be culled before incurring further expense.

Examination of the Reproductive Organs

Testes and Epididymides

The testes and epididymides should be examined for palpable gross abnormalities. Testicular tone and symmetry should be assessed. Swellings, atrophy, and lack of tone or symmetry often indicate pathologic problems and decreased fertility. The caput (head), corpus (body), and cauda (tail) of the epididymis are readily palpable in the

normal ram. The most commonly encountered epididymal lesions usually involve enlargement of the epididymis due to inflammation and fibrosis. The major infectious agents resulting in epididymitis are *B. ovis* in mature, sexually exposed rams, and *Histophilus* spp. or *Actinobacillus* spp. in young, virgin rams. Other organisms, such as *Corynebacterium pseudotuberculosis*, can also produce epididymitis.

The circumference of the two testes and scrotum should be measured using a scrotal tape. This measurement is an estimate of potential sperm production. The Western Regional Coordination Committee on Ram Epididymitis and Fertility recommends that ram lambs over 150 lb have a scrotal circumference (SC) of greater that 30 cm. And yearling rams (12–18 months) should measure greater than 33 cm. Producers of seed or breeding stock should set higher standards. Rams weighing 250 lb or more should have a SC greater than 36 cm.

Scrotal circumference varies with season.[5] The SCs recommended here are those expected during the breeding season but before the ram has been used for breeding. At this time, the testicles are their largest and should feel turgid. Soft, flaccid testes on a ram that has not been used for breeding are highly correlated with poor semen quality.

The production of spermatozoa takes place in the seminiferous tubules of the testes. The functions of the testes are regulated by gonadotropins, which are released into the bloodstream by the pituitary gland the primary external stimulus that affects the release of gonadotropins is the amount of daylight. In most breeds, a decrease in the amount of daylight is followed by an increase in the secretion of gonadotropins and an increase in sexual activity. Scrotal circumference can be 2 to 3 cm smaller and semen quality poorer as daylight hours increase and hormone levels decline. The testes can also show a decrease in size and tone following an active breeding program, as spermatozoa stores are repeatedly depleted. Depending on breed, duration of breeding exposure, environment, and season, testicular size and tone are generally regained following 30 to 45 days of sexual rest.

Prepuce

The prepuce should be free of any raw or ulcerative lesions at the orifice. Lesions can indicate conditions such as pizzle rot (sheath rot, ulcerative posthitis), lip and leg ulceration, or phimosis.

Penis

The penis should be extended and examined. If the penis cannot be extended, a previous injury or disease may have produced adhesions. The glans and urethral process should also be examined. Ulcerative dermatosis and urethral calculi can destroy the glans.

Semen Evaluation

A semen evaluation should be conducted only after the ram has been determined to be free of *B. ovis,* has passed a serving capacity test, and has passed a structural and physical examination. A listing of recommended equipment and supplies, including manufacturers, for the collection and evaluation of ram semen is provided in Table 85-5.

Semen Quality

Ram fertility can be compromised by a wide variety of infectious and noninfectious conditions. Some of the primary infectious agents that affect semen quality, including *B. ovis, Histophilus* spp., *Actinobacillus* spp., and *C. pseudotuberculosis,* have already been mentioned. Others include the blue tongue virus and any agent causing an elevation in body temperature. The noninfectious conditions affecting semen quality are generally stress related. These stressors tend to be environmental (e.g., extreme temperatures, high humidity, transportation, and nutritional compromise). Increased morphologic abnormalities of sperm cells are evidence of an insult on the reproductive tract. In many instances, it is difficult to determine whether such abnormalities are transient or permanent in nature.

Spermatogenesis in the ram requires approximately 49 days. An additional 10 to 14 days are required for the spermatozoa to travel through the epididymis. During passage through the epididymis, maturation of the spermatozoa continues and several functional changes occur. These changes include the potential for sustained motility, the progressive loss of water, the distal margination and eventual loss of the cytoplasmic droplet, and development of the potential capacity to fertilize ova.[6] Thus, semen collected on any given day was produced 59 to 63 days earlier. Therefore, the semen evaluation portion of the BSE should be conducted as near to the start of the breeding season as possible.

Collection Technique

1. Insert the lubricated probe into the rectum and put pressure on the accessory reproductive glands by pulling up slightly on the handle of the probe. Massage the seminal vesicles firmly with four to six strokes using the probe. This provides slight stimulation to the ram. Leave the probe in place.
2. Extend the penis by grasping the penis in front of the scrotum. Push the penis forward while bringing the prepuce back. Grasp the glans with a clean gauze sponge. Extend the penis and secure it by grasping the shaft of the penis immediately behind the glans using the other end of the clean gauze sponge.
3. Place the tip of the penis and the urethral process into a warm 17 × 100 mm plastic tube. It is essential that the semen sample be collected in as clean a manner as possible to prevent contamination from the glands or prepuce. Cells and cellular debris from contamination may interfere with proper evaluation.
4. With the rectal probe already in place, apply pressure on the accessory reproductive glands by pulling up slightly on the handle of the probe. Massage the seminal vesicles firmly with six to eight strokes using the probe. This massage is critical to obtaining a good collection. With steady pressure applied to the seminal vesicles

Table **85-5**

Equipment and Supplies for Collection and Evaluation of Ram Semen

Instrument or Material	Comments
Microscope	A phase contrast microscope is recommended. A light microscope with good optics can be adequate, although defects of the aerosomal cap are more clearly delineated under phase contrast. The optics for either type of microscope should include 10× eyepieces with a 10× or 20× objective for observing motility and white blood cells, plus a 100× oil immersion lens for evaluating morphology.
Electroejaculator[a,b]	A hand-held ram ejaculator is ideal. However, if only a few rams are to be examined each year, ram probes that attach to the larger bull ejaculator are satisfactory.
Slide warmer	Any good slide warmer with temperature regulation is adequate. Temperature should be from 37–38°C for the slide and tube warmer.
Tube warmer	A number of inexpensive tube warmers are available or some type of block holder can be devised in conjunction with the slide warmer.
Lubricant[c]	Carboxymethylcellulose is an excellent, reasonably priced rectal probe lubricant. Any lubricant, e.g., obstetrical lubricant, is satisfactory. When doing multiple examinations, it is advisable to mix the lubricant with a disinfectant.
Scrotal tape[a,d]	
4 × 4 Gauze sponges	Used to secure the extended penis for examination and semen collection.
Test tubes	Plastic 17 mm × 100 mm nonsterile disposable test tubes without caps work well for collecting the sample. Whirl Pak or 3 × 5 Col-Palmer Minigrip Reclosable Bags are also satisfactory and can be placed directly on the slide warmer.
Stain[a,d]	Eosin-nigrosin stains are generally used for evaluating morphology. The most common are Blom's and Hancock's.
Cell counter	An eight-place cell counter used in hematology is ideal for doing morphology counts.
Diluent	Lactated Ringer's solution, 2.9% buffered sodium citrate or 0.9% saline can be used.
Transfer pipettes	5½" Pasteur pipettes or plastic bulb pipettes work well for transferring diluent, stain, and semen.
Microscope slides	25 × 75 mm microscope slides (frosted ends are helpful).
Cover slips	18 × 18 mm or 22 × 22 mm are recommended.
Oil	Non-drying type A or B immersion oil.
Miscellaneous	For example, lens cleaner and lens paper, marking pens for labeling samples, pens/pencils, evaluation forms, paint sticks, spare microscope bulbs, extra batteries for ejaculator.

[a]Lane Manufacturing Co, 2075 S. Valencia, Denver, CO 80231.
[b]Western Instrument Co, PO Box 16428, Denver, CO 80216.
[c]Burns Veterinary Supply, 6804 E. 48th Avenue #B, Denver, CO 80216.
[d]Trueman Manufacturing, Box 774, Edmonton, Alberta, Canada T5J 2L4.

using the probe, apply electrical stimulation to the ram for 8 to 10 seconds. Release the stimulus and wait until the ram completely relaxes (this may be 10–12 seconds), then apply six to eight massage strokes, again with pressure on the seminal vesicles. A second electrical stimulation for 8 to 10 seconds may be necessary and usually produces a good ejaculate. Observe whether the semen comes from the tip of the penis, the urethral process, or an opening in the urethra along the shaft of the penis. An old urolithiasis lesion on the urethra, midway up the penis, may allow the semen to be misdirected during breeding.

Poor technique often results in poor collection that contains mostly seminal fluid and very few spermatozoa. Inadequate stimulation resulting from weak batteries in a handheld ejaculator will also produce a poor, thin ejaculate. Applying too much voltage is the most common mistake when a large bull electroejaculator with a ram probe is used for collection. If stimulation is too severe, rams tend to urinate or lose their erection.

Microscope Evaluation

The semen sample *must* be maintained at 37°C!

Motility. With a Pasteur pipette, transfer a tiny drop of semen onto one end of a warm microscope slide. On the other end, place a drop of warm (37°C) diluent (e.g., saline, lactated Ringer's solution, or 2.9% buffered sodium citrate). With the corner of a coverslip, transfer a small amount of semen to the diluent, mix, and place the cover slip over the diluted semen. Inadequate dilution is a common error. Using a 10× or 20× objective, observe a number of fields and evaluate *progressive* motility of individual spermatozoa. If a field is too crowded, individual spermatozoa cannot be evaluated and an accurate estimate of progressive motility *cannot* be made. A good sample should have greater than 40% progressively motile sperm. Motility can be affected by a number of extraneous factors, such as urine contamination, cold shock, and the number of stimulations required to obtain a collection. Therefore, motility alone is not always a good indicator of potential fertility.

White blood cells. When observing motility, the presence of white blood cells (WBCs) should also be noted. WBCs appear as round, shiny, or refractory objects. Under

increased magnification, the nucleus can also be observed. Although WBCs can be observed using a standard microscope, they are seen more clearly with a phase contrast microscope. The presence of WBCs indicates inflammation or infection of the reproductive tract. It is not uncommon, however, to observe an occasional WBC in a sample contaminated by preputial fluids. In older rams, WBCs can signal *B. ovis* infection. In young virgin rams, WBCs often indicate *Actinobacillus* spp. or *Histophilus* spp. infection.* A ram with greater than 10 WBCs per 20× field and no palpable lesions should be considered for treatment and re-evaluated. If *B. ovis* is indicated in a mature ram, the animal should be culled.

Morphology. With a Pasteur pipette, transfer a small drop of semen onto the end of a microscope slide. Adjacent to the drop of semen, a ribbon of eosin-nigrosin stain is placed across the slide. With the center of a second slide, pick up a small sample of semen and mix it with the stain (similar to a blood smear for staining). The slide is allowed to dry, and observed under oil immersion. Count 100 spermatozoa and record the number of normal sperm, as well as the number and type of abnormal sperm. The percentage of normal spermatozoa is of foremost importance; however, noting the types of abnormalities observed in a poor-quality sample may provide information as to the cause and recovery potential. Figure 85-1 is an example of normal sperm morphology.

Common morphologic defects

DETACHED HEADS. Detached or separated heads (Fig. 85-3*A*) are frequently observed in conjunction with testicular degeneration. This may be a transient condition, as in the case of heat stress or a mild frostbite, or a permanent condition, as in the case of *B. ovis* infection. Figure 85-2 is typical of an ejaculate obtained from a *B. ovis* infected ram.

HEAD DEFECTS. Misshapen heads (Fig. 85-3), pyriform heads (Fig. 85-4*B*), macrocephalic heads, and microcephalic heads (Fig. 85-4), are all associated with testicular hypoplasia or mild degeneration.

ACROSOMAL DEFECTS. Acrosomal defects (Fig. 85-5) can have a major impact on fertility. Knobbed, flattened, dented, ruffled, and missing acrosomes are not uncommon and are generally associated with testicular degeneration. There is also some evidence that the knobbed defect may have a hereditary basis.[7]

CYTOPLASMIC DEFECTS. Cytoplasmic droplets generally migrate down the midpiece and are shed as the sperm cells mature in the epididymis. Unshed droplets are generally in a proximal (Fig. 85-6) or distal (Fig. 85-7) position on the midpiece. Proximal droplets are associated with immaturity, periods of low hormonal activity, overuse, and testicular degeneration. Cytoplasmic droplets are frequently associated with bent and coiled tails.

TAIL DEFECTS. The distal midpiece reflex (Fig. 85-4*C*) is the most common tail abnormality. A cytoplasmic

*Unlike *B. ovis* infection in mature rams, *Actinobacillus/Histophilus* infection can generally be cleared up with tetracycline therapy. For treatment to be effective, it must be initiated before permanent damage has occurred. A therapeutic level of tetracycline must be maintained for approximately 6 days.

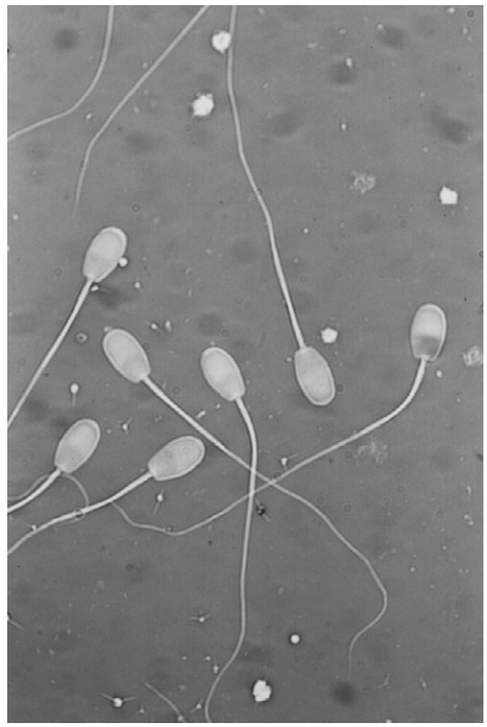

Fig. 85-1 Normal ram sperm morphology.

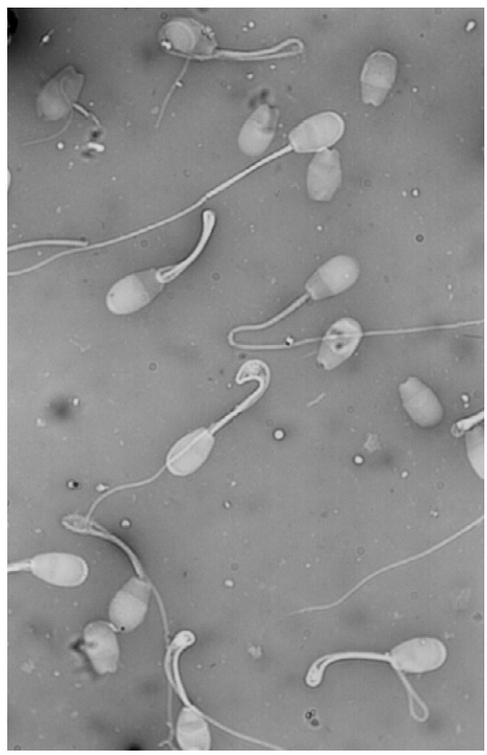

Fig. 85-2 Semen sample from a ram infected with *Brucella ovis*.

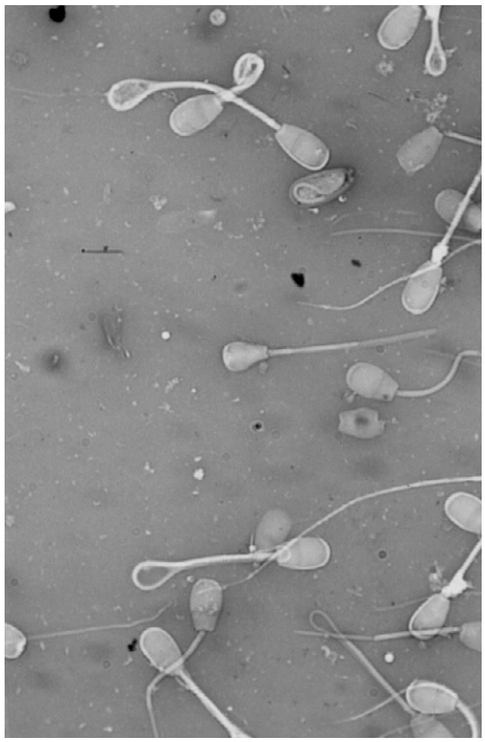

Fig. 85-3 Head defect in ram sperm: misshapen heads.

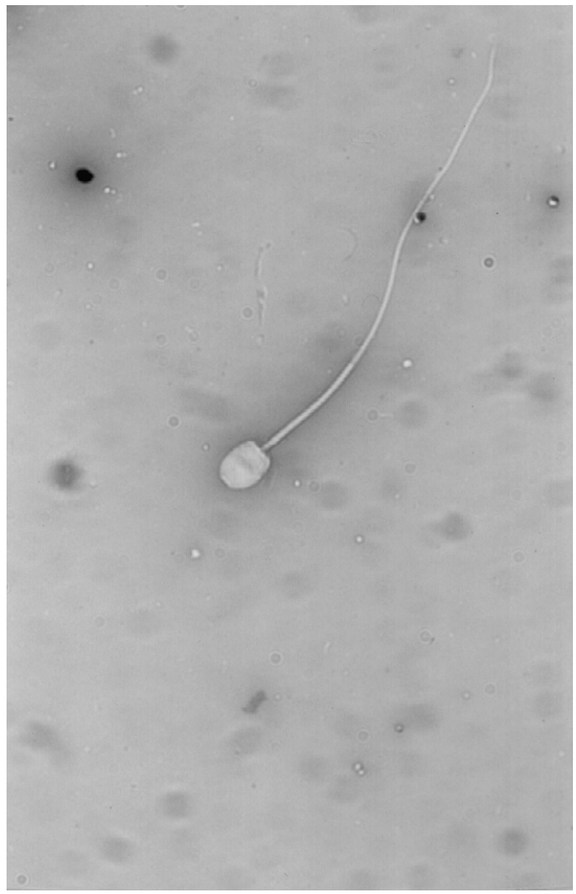

Fig. 85-4 Head defect in ram sperm: microcephalic head.

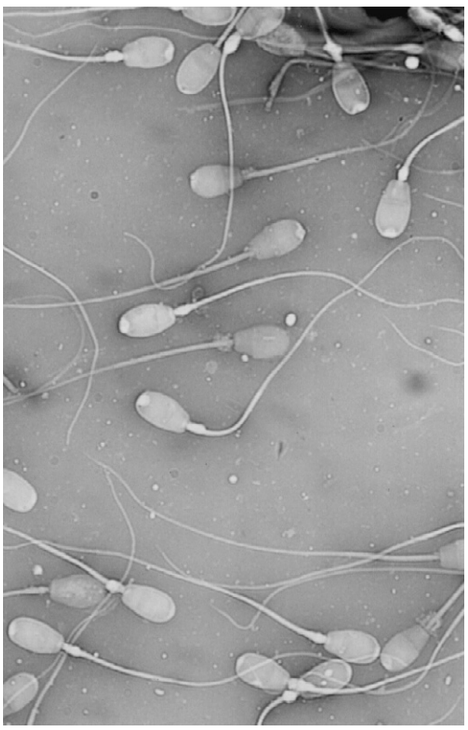

Fig. 85-5 Acrosomal defects in ram sperm.

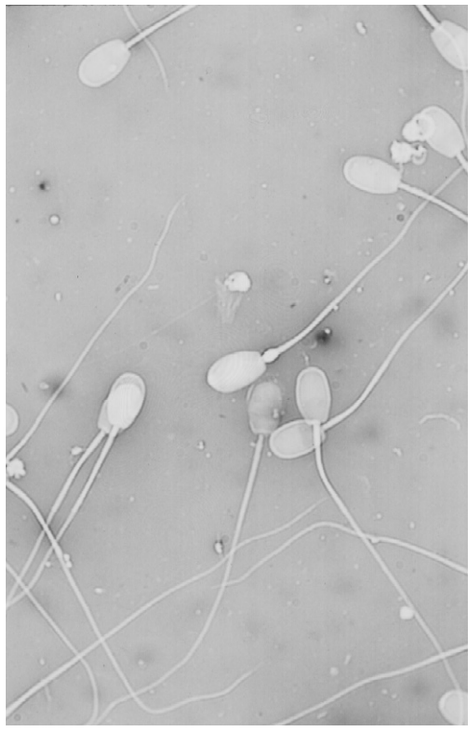

Fig. 85-6 Proximal cytoplasmic droplets from ram sperm.

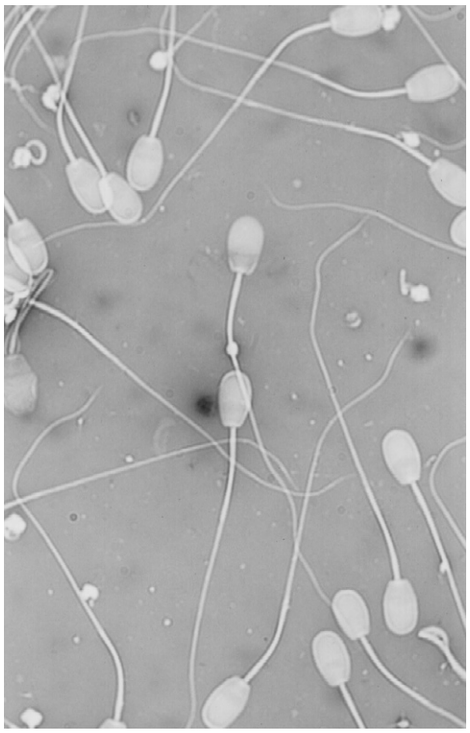

Fig. 85-7 Distal cytoplasmic droplets from ram sperm.

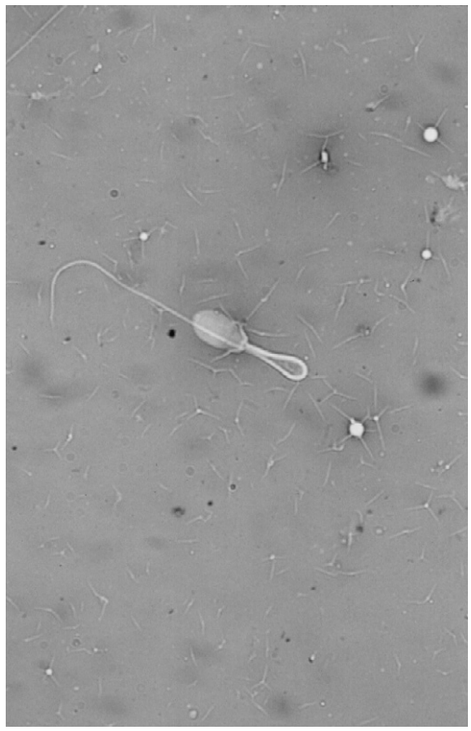

Fig. 85-8 Tail defect in ram sperm: distal midpiece reflex.

droplet is frequently trapped inside the bend. Sperm with a distal midpiece reflex appear to be swimming backward. Trace minerals have been found to play an important regulatory role in development and maturation of certain sperm tail components.[8] Tightly coiled tails (Fig. 85-8) are frequently associated with testicular degeneration.

ARTIFACTS. Improper staining technique can produce artifacts of sperm abnormalities. For example, incorrect stain osmolality can result in numerous reflex midpieces.

Classification

Table 85-6 presents recommendations for classifying the potential breeding capacity of a ram. These recommendations assume that the ram is *B. ovis* free, has passed a structural and physical examination, and displays good libido.

SURGICAL STERILIZATION

Vasectomy

Vasectomy can be performed using an epidural block of lidocaine for regional anesthesia. The skin area on the posterior portion of the scrotum, as it joins the body, is aseptically prepared. Assuming the surgeon is right-handed, he or she grasps the cord (which includes the cremaster muscles, pampiniform plexus, vas deferens, veins, artery, and nerves) (Fig. 85-9) with the left hand. By grasping the cord tightly between the fingers and thumb, one

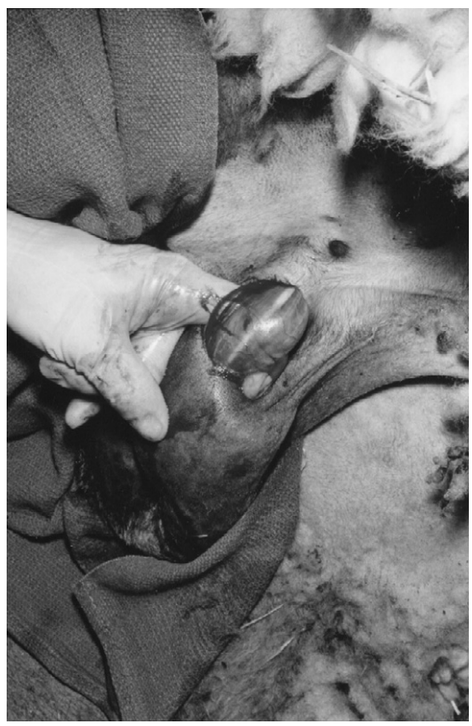

Fig. 85-9 Spermatic cord of the ram, grasped in preparation for vasectomy.

Table **85-6**

Recommendations for Classifying the Potential Breeding Capacity of Rams

	Excellent	Satisfactory	Questionable	Unsatisfactory
Ram Lambs, 6–12 mos	SC > 33 cm Mot > 50% >90% Normal sperm	SC > 30 cm Mot > 30% >70% Normal sperm	SC < 30 cm Mot < 30% <70% Normal sperm	SC < 30 cm Mot < 30% <50% Normal sperm
Yearlings, 12–18 mos	SC > 35 cm Mot > 50% >90% Normal sperm	SC > 33 cm Mot > 30% >70% Normal sperm	SC < 33 cm Mot < 30% <70% Normal sperm	SC < 33 cm Mot < 30% <50% Normal sperm

Mot, motility; SC, scrotal circumference.

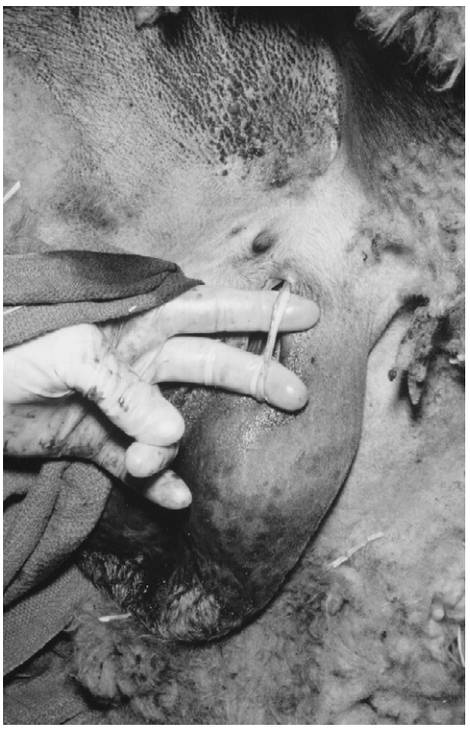

Fig. 85-10 Isolation of the spermatic cord in the ram, for vasectomy.

can roll the cord and identify the vas deferens. Using a scalpel, the surgeon cuts down onto the vas deferens, identifies the structure, and isolates it by blunt dissection. A nonabsorbable ligature is placed at the proximal and distal portions of the exposed vas deferens, and a 1- to 2-cm section is removed (Fig. 85-10). The wound is closed with an appropriate skin closure technique.

Epididymectomy

An epididymectomy is the removal of the tail portion of the epididymis. This operation is performed under regional anesthesia as described for vasectomy. The skin of the scrotum is again aseptically prepared. The surgeon grasps both testes and forces them firmly into the ventral portion of the scrotum incising the skin that overlies the tail of the epididymides to expose the epididymis. Using surgical scissors, the surgeon separates the tail of the epididymis from the body of each testis. This separation should extend to the body of the epididymis on the medial aspect of each testis. A nonabsorbable ligature can then be placed around the body, and the tail of each epididymis can be removed. Again, an appropriate skin closure is used.

Caution! Complete reproductive sterility of the ram is not immediate following surgery. Success should be verified by microscopic examination of an ejaculate collected 60 days after surgery.

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CHAPTER 86

Artificial Insemination and Embryo Transfer in Sheep

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Artificial insemination and embryo transfer in sheep offer many advantages for genetic improvement. They are not new techniques, but several advances in technology have made them somewhat easier for cost-effective implementation. At this time, many of the drugs that are routinely used for synchronization and superovulation are not available to U.S. veterinarians, yet they are commonly used in most foreign countries.

ARTIFICIAL INSEMINATION

Benefits

Artificial insemination (AI) provides many benefits to the producer. They include, but are not limited to, the following:

- Increased genetic gain by allowing increased selection pressure on the ram population to hasten genetic improvement
- Increased offspring from a superior sire
- Availability of a superior sire to small flocks
- Frozen and fresh semen that can be shipped and stored and moved nationally or internationally with fewer health risks and welfare implications than live animals
- Disease control through testing of rams before use and treatment of the semen with antibiotics to lessen the threat of disease
- Semen that can be frozen as insurance against injury or death of a superior sire
- Sire evaluations that can be carried out in many environments to assess the superiority of that sire over many different production types

Selection and Management of the Ram

The ram should have a complete physical examination and be superior in genotype and phenotype. He should be in good body condition (body condition score [BCS] 3–3.5) and be evaluated for locomotion. The reproductive tract should receive special attention and the prepuce examined for lesions and discharge. Testing for any diseases should be done at this time.

The penis should be manually examined for defects or injuries. This can accomplished by setting the ram up on its dock and pushing on the sigmoid flexure while pushing back on the prepuce. There should be no scars, adhesions, or abrasions and the urethral process should be intact.

The scrotal circumference should be measured and compared to normal values for age, breed, and season. Minimum circumferences should be per age and size and carefully adhered to. Sperm production is linked closely to testicle size, as is pubertal age of the offspring.

The testicles should be palpated in a routine fashion so that no abnormalities or defects are missed. The testicles themselves should be firm with no palpable hard or fluctuating lesions that might suggest neoplasm, trauma, abscess, or venous damage. The head, body, and tail of the epididymis should be palpated as well for any lesions with particular attention to the tail of the epididymis and the spermatic cord where varicoceles and sperm granulomas may be found.

The scrotum should be symmetrical and not have any scars, adhesions, or evidence of parasites and it should hang low to help maintain a scrotal temperature 4° to 7°C below body temperature. There should be no signs of scrotal hernia, cryptorchidism, testicular hypoplasia, or other defects. Any such abnormality should exclude the ram from use as an AI sire.

Libido testing should be done if possible. Rams should mount and breed freely and have no feet abnormalities.

All stressful management procedures such as shearing, deworming, vaccinations, vitamin and mineral injections, and hoof trimming should be done 6 to 8 weeks before collection to avoid causing damage to sperm during the spermatogenic cycle.

The rams should be on a rising plane of nutrition and be fed ad lib good quality hay or forage as well as protein and concentrate that is low in Mg^{2+} and Ca^{2+} and may contain 1% ammonium chloride to help prevent urolithiasis. Depending on the area of the country and the quality of forage and concentrate available, supplementation of vitamin E and selenium may be advisable. Low blood copper and zinc have been implicated in reduced libido and fertility, and assessment of these in the diet or blood may be advisable.

Exposure of rams to cycling ewes at this time may also help semen production, especially in rams from more seasonally dependent breeds.

Two weeks before semen collection, rams should be individually housed to help prevent injury, avoid homosexual behavior (especially in ram lambs), and help control any special dietary needs of individual rams. This may also help more submissive rams increase their libido. All contact with ewes should stop at this time, but it is

important to maintain ewes within sight, smell, and sound of the rams.

Although semen may be collected at any time of year, it is important to note that many of the breeds are seasonal and their semen quality and quantity are affected when collected out of the normal breeding season. To this end, it is important to collect those rams during the natural breeding season even if the semen is to be used out of season.

RAM TRAINING FOR SEMEN COLLECTION WITH THE ARTIFICIAL VAGINA

Rams can be trained to mount a teaser ewe and ejaculate into an artificial vagina with a collector kneeling beside him. Many factors affect how long this training will take, but age of ram, breed, season, libido, mating experience and temperament all affect training time. Most mature rams will mate an ewe restrained in a standing position. Ejaculates may be collected daily from healthy mature rams, but sperm concentration and quality should be evaluated on a daily basis and collections adjusted accordingly to achieve optimal use of the ram.

Rams with low libido or inexperience may be taught to mount and collect by following these guidelines:

- Move the rams to the collection area or similar surroundings 2 to 4 weeks before collection and accustom them to hand feeding, gentle handling, and the presence of humans.
- Introduce one or two estrus ewes into the pen and allow the rams to mount normally. Teaser ewes may be made for such use by ovariectomizing them and giving 1 mg of estradiol benzoate once weekly. If two ewes are kept and injected on a Monday/Thursday morning schedule, at least one of them is available throughout the week.
- Let the ram mount a ewe that has been restrained in a head catch with a handler in close proximity.
- After the ram is comfortable with mounting, the handler then starts to gently touch the sheath upon mounting to condition him to handling. Techniques such as changing the ewe, allowing the ram to watch another ram mount, or allowing a ram to choose a ewe may help the slower, more timid ram to mount. Short periods of training are preferable to longer periods and direct contact between rams should be avoided.
- Once regular mounting is achieved, the handler diverts the penis into the artificial vagina (AV) for collection by moving the sheath. Patience and gentle handling go a long way to improving collection and performance of the ram.

The Artificial Vagina

The AV is made up of a liner inside a hard casing that is firmly held in place with rubber bands. These can be purchased commercially or homemade but must be watertight because sperm is killed by any direct contact with water. A stopcock or other device is drilled through the casing to add water and air to control the temperature and the pressure. A glass or plastic collection vessel is attached to one end and a cover or sleeve is applied over it to prevent cold shock to the semen. The open end is lubricated with a nonspermicidal sterile obstetric gel.

Each ram should have his own AV and liner, which should be cleaned with purified water and then rinsed with alcohol and let air-dry between uses. Collectors should wear clean vinyl examination gloves for handling and collecting semen.

Just before use, the AV should be filled half full with water that is 50° to 55°C and the collection container inserted into one end. Correct pressure is attained by blowing air into the AV through the control valve and is easily evaluated by inserting a clean gloved finger into the AV through the open, lubricated end. Exact pressure and temperature will vary between rams, but a final collection temperature (internal) of 40° to 45°C and a pressure that allows easy entry of the penis but still allows for moderate pressure will usually work best.

Semen Collection

The ram will approach the restrained ewe and the collector should be positioned near the ewe, ready to collect with a properly prepared AV. The ram will normally smell, lick, and paw at the ewe, who will respond by urination, vocalization, and tail deviation. Time to mount will vary, but well-teased males tend to produce more semen per collection. When the ram mounts, the collector should deflect the prepuce of the ram to the side, allowing the penis to deviate into the AV. The AV should be applied in such a way that the ram's ejaculatory thrust is not impeded in an upward manner. The penis itself should not be handled.

Problems that may occur with collection with the AV include failure to ejaculate due to improper AV temperature or pressure or improper collector technique; delayed ejaculation after the AV is removed, usually due to poor technique or to over- or understimulation; and lastly, premature ejaculation due to poor technique, high libido, or overteasing.

Once trained, most rams are easy to handle for collection of semen and the ejaculate is superior to that obtained from electroejaculation because it usually does not contain excessive accessory gland secretions.

Electroejaculation for Semen Collection

Electroejaculation of the ram is a relatively simple and easy procedure with the correct equipment. In rams that are going to be used extensively in an AI program, it cannot be recommended because of the repeated stresses on the animal, but for minimal collections or animals that are too debilitated to mount a teaser, it is a viable alternative for the practitioner. It is also useful when examining a large number of rams in a day, for collection of vasectomized rams before use or for rams that refuse to serve an AV. It has also been observed to increase libido in some low-libido rams.

Most common equipment includes either a Bailey or a Ruakura ram probe, although other probes by other manufacturers will also work. These types of probes have a

simple on/off switch that delivers stimulation of 10 to 15 volts of 30 to 50 sine or square waves. Operators should thoroughly familiarize themselves with whatever equipment they are using and use it appropriately for the situation.

Rams may be collected with or without sedation depending on their disposition, skill of the operator and assistants, as well as the frequency and objectives of the collection. Xylazine (15–20 mg IM) or acepromazine (10 mg IM) have been very effective in the authors' experience. The ram should be restrained in lateral recumbency and the penis exteriorized (may be easiest to accomplish while the ram is set up on its dock and then lowered to lateral recumbency). The penis is grasped just below the glans with a sterile gauze sponge and the glans and urethral process directed into the collection container. Care must be taken not to cold shock the semen, so either a cover or water bath around the collection vessel is appropriate. The penis and prepuce may be cleaned and clipped at this time to decrease chances of contamination of the semen. Phosphate buffered saline (PBS) or saline may be used so if contamination with them occurs, it will not affect semen. The rectum is then cleared of any feces by a gloved hand and lubrication applied liberally to the probe and anus. Gentle massage of the accessory glands with either the fingers or probe will help to stimulate the ram and may ease collection. Generally this is done for 10 to 15 seconds and then stimulation applied with the probe in a rhythmic on/off sequence of 3 to 5 seconds on and 5 to 15 seconds off with gentle downward pressure toward the pelvic floor. Gentle movement of the probe during the on/off sequence may help the collection and help to find the proper depth of the probe for maximum stimulation. Individual rams vary in their response, but generally, ejaculation occurs after 3 to 5 stimulations and if collecting for freezing and maximum sperm numbers are desired, stimulation may continue for several more stimulation sequences until ejaculation is not apparent or sufficient semen is obtained. If no ejaculation occurs after several stimulations, the probe should be removed and cleaned of feces and the battery checked on the electroejaculator. Having several collection containers available may help to fractionate the sample so only the sperm-rich semen is collected with minimal accessory gland contamination or, in some cases, urine. Encouraging the ram to urinate before collection is also helpful to eliminate or reduce the chance of contamination with urine. Any accessory fluid that is collected should be discarded, or in some cases, the semen may be centrifuged so that the accessory fluid can be discarded. Once collected, the semen is handled and examined as in AV collection.

HANDLING, EXAMINATION, ASSESSMENT, AND EVALUATION OF SEMEN

Semen must be handled carefully to avoid heat shock; cold shock; contamination with water, disinfectants, sunlight, and air; and any other process or product that may decrease viability. Sperm will die if temperatures exceed 45°C and any increase above 37°C will increase metabolic rate and thus decrease sperm life. Cold shock may be avoided if all equipment that semen comes into contact with is kept at 30° to 37°C. All containers that come into contact with the semen should be either plastic or glass and cleaned with laboratory type cleaners, or in the authors' opinion, single-use containers are preferred, if practical.

Semen should be examined as soon as possible after collection and transferred to a sterile collection tube held in a 30°C water bath. The criteria used for a breeding soundness examination of rams should be followed in the examination and evaluation of the semen. Color should be milky off-white. Pink usually indicates contamination with blood, and a gray or brown color may be indicative of a reproductive tract infection. Urine contamination is usually yellow with a characteristic odor and the semen will appear dilute. Any off-quality semen should be discarded and not used for AI.

Volume will range from 0.5 to 1.5 ml and may vary based on frequency and type of collection. Repeated collections over several days will decrease volume. Motility will normally be wave-like on gross examination under low power (10–50×) on a prewarmed slide without a coverslip. The motion is scored as in Table 86-1 and any sample scoring 2 or below is discarded.

Concentration should be calculated accurately because a reliable number of sperm per insemination is critical to pregnancy rates. An average ejaculate will contain 3.5 to 6.0 billion sperm per milliliter. A hemocytometer, spectrophotometer, computer-assisted analysis, or other accurate method of counting sperm is best, but under some field conditions, concentration of the sperm may be estimated by consistency of the sample using Table 86-2. Thick, creamy samples contain more sperm than those of a more dilute, watery consistency.

Other tests that may be useful include a morphologic and acrosome integrity test using eosin-nigrosin stain or

Table **86-1**

Scoring System for Wave Motion Under Low-Power Microscopy, 10× to 50×

Score	Class	Description
5	Very good	Dense, very rapidly moving waves; individual sperm cannot be observed; >90% of the sperm are active
4	Good	Vigorous movement, however waves and eddies are not as rapid as those in score 5; 70 to 90% of the sperm are active
3	Fair	Only small, slowly moving waves; individual sperm may be observed; 40 to 65% of sperm are active
2	Poor	No wave motion forming, but some movement of sperm visible; 20 to 40% of sperm are active
1	Very poor	Less than 10% of sperm active; possible to observe slight "flickering" of sperm with poor motility
0	Dead	No movement apparent

hypo-osmotic swelling tests. Individual sperm motility and progressive forward movement by dilution of the sample with extender, sodium citrate, or phosphate buffered saline. Longevity of sperm using various extenders and examination at set intervals (usually 1–2 hours) over time may be used to evaluate both the sperm and which extender gives greatest sperm life. Temperature and holding conditions must be carefully monitored to be sure that the sperm is not affected by outside factors and the evaluation is valid.

DILUTION AND INSEMINATION DOSE USING FRESH SEMEN

During natural mating, a mature fertile ram normally deposits approximately 3 billion sperm. Of these, only 100 million to 150 million penetrate the cervix and get into the uterus. Doses of semen containing 100 million sperm have achieved acceptable conception rates when deposited into the cervix. If undiluted, this small volume is very hard to handle and measure accurately. Dilution not only solves this problem, it provides the sperm with a suitable environment for preservation during the insemination and handling process.

Various extenders are available for fresh dilution, but the two most commonly used are heat-treated cow milk or Dulbecco's phosphate buffered saline (with or without 2% equine serum albumin or 10% fetal calf serum). Ultra-high temperature milk may be purchased or made by heating whole milk to 92° to 95°C for 8 to 10 minutes. Water must not splash into the milk, which must not be allowed to boil. The extender must be either cooled or warmed to 30°C and then added slowly to the extender as soon as possible after collection and used as soon as practical (within 1 hour).

Table **86-2**

Concentration of Ram Semen Assessed for Consistency

		Number of Sperm ($\times 10^9$)	
Score	**Consistency**	***Mean***	***Range***
5	Double creamy	5.0	4.5–6.0
4	Creamy	4.0	3.5–4.5
3	Milky	3.0	2.5–3.5
2	Thin milky	2.0	1.0–2.5
1	Cloudy watery	>1.0	0.3–1.0
0	Clear (watery)	Insignificant	0.3–1.0

Note: Score 0 to 2 semen should not be normally used for insemination.

Freezing Semen

There are many protocols for freezing ram semen. One-step and two-step methods have been described, but because of its ease, the one-step method is the most commonly used. Semen is diluted to the final prefreezing dilution rate at 30°C with the cryoprotectant. Straws are then loaded and cooled slowly (using a water bath) by placing them in a refrigerator and cooling to 5°C over 1.5 to 2 hours. One formula that can be used is tris(hydroxymethyl) aminomethane (24.2 g) plus citric acid (13.6 g) plus fructose (10.0 g) plus glycerol (64 ml) plus egg yolk (200 ml) in sufficient quantity to produce 1 L at a pH of 6.8. Commercial extenders have been used with success by the authors and for small numbers and ease of use are very satisfactory.

Freezing semen in straws is similar to freezing bull semen, but the extender, dilution rates, cooling rates, and other factors will vary from laboratory to laboratory. Ram semen is more difficult to freeze than bull semen and some rams (5–10%) will not freeze successfully with current techniques and extenders that are available. Normally ram semen is diluted to at least 1:8 with the chosen extender with final dilution depending on the type of insemination (laparoscopic AI versus cervical AI versus transcervical AI). Doses of semen for the various techniques can be found in Table 86-3.

Semen can be loaded into straws or cooled before loading into straws, then cooled slowly to 4° to 5°C over a period of 1.5 to 2 hours. Depending on the laboratory or extender, semen is then either frozen over nitrogen vapor (4 cm for 0.25-ml straws or 6 cm for 0.5-ml straws) on a chilled (5°C) rack or held for several more hours to allow equilibration and then frozen as described previously. After 8 to 10 minutes in the vapor, the semen is then plunged into the liquid nitrogen and from there loaded into canes and transferred to a tank for storage using standard techniques. When using a programmable

Table **86-3**

Typical Dose Rates and Inseminate Volumes Required for Different AI Techniques

Technique	Type of Semen	Volume, ml	Required Dose of Progressively Motile Sperm	Concentration of Inseminate/ml
Cervical	Fresh only	0.2	Min 200×10^6	1000×10^6
Vaginal insemination	Fresh only	0.2	Min 400×10^6	2000×10^6
Transcervical intrauterine	Fresh or frozen	0.5	$50–100 \times 10^6$	$200–400 \times 10^6$
Laparoscopic intrauterine	Fresh or frozen	0.05	$20–40 \times 10^6$	$400–800 \times 10^6$

Min, minimum.

freezer, chilled filled straws are loaded into the freezer and cooled at the following rate: 4°C per minute until semen reaches –12°C, then 40°C per minute from –12° to –40°C, then finally 50°C per minute from –40° to –140°C. After this point is reached, straws are loaded into canes and stored as described previously.

Semen can also be frozen in pellets by using a block of dry ice into which small depressions have been punched using a metal rod or die for multiple depressions. Semen frozen in pellets is usually diluted to only 1:3 to 1:4, depending on sperm evaluation. Then 0.1 to 0.15 ml of semen is pipetted into each depression and after 2 to 3 minutes the pellets are transferred to plastic goblets, labeled, and put in liquid nitrogen. For thawing, pellets are put into an extender prewarmed to 40°C, or two to three pellets are placed in a sterile dry test tube or Whirl Pak bag and put in a water bath at 40°C and shaken or stirred vigorously till the pellets melt. The semen is then transferred to a 30°C water bath, where it may be held for up to 18 hours, till insemination. This technique does not require expensive or sophisticated equipment, but labeling and identity of the semen are harder to maintain.

Frozen ram semen can be expected to achieve conception rates of 65% when using laparoscopic intrauterine AI.

EWE SELECTION AND MANAGEMENT

The success of the AI program depends as much on the successful management of the ewes as it does on the other techniques mentioned previously. Ewes must be in good health and condition and be free of any diseases. They should be neither fat nor thin; have sound feet, legs, udder, and teeth; and be free of heritable faults. Lambs should be weaned at least 6 to 8 weeks before insemination, and stressful procedures such as shearing, vaccination, and deworming should also be avoided during this time as well as for 4 weeks after insemination. Best conception rates and lambing percentage will be obtained when ewes are flushed (increasing calories and protein) 2 to 3 weeks before breeding. Identification of the ewes and accurate records are very important, especially when multiple sires are being used. Best conception rates are obtained when breeding during the natural breeding season.

SYNCHRONIZATION AND DETECTION OF ESTRUS

To achieve optimal pregnancy rates, it is imperative to time insemination to estrus and ovulation. Accurate detection of estrus in the ewe for insemination using a marking harness on a teaser ram can be used to detect heat with the expectation of 6% of ewes to be in heat each day over a 17-day estrous cycle.

Synchronization programs for AI, especially for out-of-season breeding, may make utilization of time, personnel, and equipment more efficient. This will reduce the time spent in a program and allow for selection of the optimal number of ewes to be inseminated each day. The following is a typical program:

Day 0: progesterone started (CIDR [controlled intravaginal drug release] or sponge or Synchromate implant)
Days 12 to 14: progesterone withdrawn and pregnant mare serum gonadotropin (PMSG) (300–600 IU depending on breed and season)
+ 24 hours: teasing by vasectomized ram
+30 to 36 hours: gonadotropin-releasing hormone (GnRH), 50 to 100 µg
+48 hours: inseminate with fresh semen
+53 to 58 hours: inseminate with frozen semen

The recent addition of GnRH to the synchronization program may help pregnancy rates and give a tighter synchrony of ovulation. Using fixed-time AI, fertility may be decreased and it may be helpful, if not imperative, to present only ewes that have been marked by the teaser or seen in estrus.

METHODS OF INSEMINATION

Using frozen semen, intrauterine insemination is currently the only way to achieve acceptable pregnancy rates. Sheep have a complex cervical canal that is approximately 7 cm long with a series of 6 to 8 rear-ward facing, offset rings that make transcervical insemination difficult, if not impossible, because the cervix cannot be digitally manipulated per rectum as in the cow. Using fresh semen, vaginal and cervical deposition may result in acceptable pregnancy rates if using the appropriate dose of semen (see Table 86-3). Attempts to standardize transcervical AI using the Guelph system and an adapted rigid endoscope for AI using frozen semen have yielded less than stellar results and have fallen into disfavor.

Vaginal Insemination

Vaginal insemination using fresh or chilled semen is done after cleaning the vulva (do not use disinfectants or other spermicidal agents) and inserting the insemination pipette along the dorsal wall of the vagina to the anterior vagina and depositing the semen there. This is also referred to as the "shot in the dark," or SID, method. Pregnancy rates with frozen semen are not acceptable, but the technique is very fast; and fresh or chilled semen may yield acceptable rates, but it is a very inefficient use of semen.

Cervical Insemination

Cervical insemination is performed with the ewe restrained "over the rail" by an assistant and the use of a speculum, head light, and angled tip insemination gun. The speculum is inserted to approximately 12 cm with the jaws parallel to the lips of the vulva, then rotated 90 degrees and its jaws opened (Fig. 86-1). The cervical os is located and the tip of the insemination pipette inserted as far into the cervix as possible without using excessive force. The speculum is then withdrawn or closed, semen is deposited, and the insemination pipette is withdrawn so as to exclude any air and help keep semen backflow to a minimum. The cervical os may be hard to locate or may

Fig. 86-1 Ewe positioned over a bale. Vaginal speculum is in place and insemination pipette is inserted into cervical os.

be covered with mucus, and the ewe may have to be repositioned or mucus drained by raising the front end of the ewe to make the os visible. A human sigmoidoscope may also be used. Any urine should be drained and the vagina flushed with saline or milk before insemination if the ewe has urinated when presented for insemination.

Laparoscopic Intrauterine Insemination

Laparoscopic intrauterine insemination is performed using specialized equipment and small amounts of semen. It has a success rate of approximately 65%, but this rate will vary depending on the quality of semen, time of year, condition of the ewes, and skill of the inseminator. The main disadvantages are the need for expensive laparoscopic equipment, invasive surgery, and the technical expertise needed to perform the procedure. Cost usually limits the procedure to expensive semen, valuable animals, or breeding companies.

Ewes should be held off feed for 24 to 36 hours and off water for 12 to 18 hours to decrease rumen fill, decrease chances of regurgitation, and reduce rumen size to allow easier visualization of the uterus. Equipment, personnel, pens, and handling devices should be arranged so that the procedures are done as quickly and efficiently as possible. Ewes are typically sedated 30 to 45 minutes before insemination with 5 to 10 mg of acepromazine IM and stressed as little as possible. Ewes are restrained in a laparoscopic cradle in dorsal recumbency and securely restrained. Wool 10 to 20 cm cranial to the mammary glands is surgically clipped and the skin is prepared for surgery using standard surgical technique. A local anesthetic is injected at the two penetration sites approximately 5 to 7 cm cranial to the udder and 3 to 4 cm on each side of the midline. Care should be taken to avoid major blood vessels.

At this point, the cradle is tipped up so that the ewe is on a plane at approximately 45 degrees with the head down. The abdominal organs are displaced cranially to allow better visualization of the bladder, rectum, and uterus. The gas trocar is introduced into the abdomen through one of the anesthetized areas, and CO_2 is used to insufflate the abdomen to the satisfaction of the operator, and then the other trocar is introduced through the other anesthetized area. Both trocars are removed and the laparoscope is inserted into one of the ports and the internal organs are identified. The uterus is usually located just under the bladder or under the caudal omental fat. After the uterus is identified and moved into position if necessary, the loaded insemination pipette is introduced and directed toward the uterus midway between the bifurcation and the uterotubal junction. A quick stab is made into the lumen and one half the dose is deposited in one horn and then the procedure repeated in the other horn.

After insemination, the insemination pipette is removed, then the laparoscope. The abdomen is deflated and the cannulae removed. Bleeding can be stopped from the puncture sites with either pressure, suture, or staples. Some operators close all punctures with suture, and others only close those that need it. Prophylactic antibiotics may be given as well as protection for tetanus. Instruments should be sanitized between animals by bathing in a suitable antiseptic such as 0.5% benzalkonium chloride. An experienced team in a well-equipped and organized operation may be able to do 300 or more ewes in a day using this technique.

Transcervical Intrauterine Insemination

The Guelph system for transcervical AI (GST-AI) requires special positioning of the animal, cervical retraction and stabilization, and the use of specially designed instruments for the stabilization and passage of the insemination pipette through the cervix. These instruments are no longer commercially available to the authors' knowledge. Ewes are restrained in dorsal recumbency in a fetal-like position to ease stabilization and retraction of the cervix. A foot trimming table may work well for this as well as a specially designed crate. A Plexiglas vaginal speculum (30 mm outside diameter) with light source is lubricated and introduced into the vagina. The speculum should have a 1-cm opening along one side for instruments to pass. The cervix is identified and a pair of Bozeman forceps is introduced to grasp the tissue near the cervical os. This part of the procedure is critical and requires much expertise and training for consistent success. Correct attachment of the forceps facilitates entry and passage of the insemination equipment through the cervix. The cervix is then retracted and the handle of the forceps is passed through the side opening in the speculum so that the cervix can be better visualized. The bent-tipped preloaded insemination gun (modified Cassou) is then introduced into the cervical canal and rotated continually while attempting to negotiate through the cervical rings. Manipulation of the cervix at the same time with the forceps may improve or help passage through the rings. Once the cervical rings are penetrated, semen is deposited in the uterus, the pipette withdrawn, cervix released, and the speculum removed. Trained, experienced inseminators may penetrate the cervix in as much as 75% to 85% of ewes, more in accelerated lambing programs.

Cervical injury, abscesses, infections, and poor pregnancy rates are all associated with this technique, but vary by operator, semen dose, ewe condition, and experience. Pregnancy rates are generally lower than with laparoscopic AI, but this procedure may be a viable alternative when reduced pregnancy rates are acceptable.

FACTORS AFFECTING THE SUCCESS OF AN AI PROGRAM

Close attention to all details is critical in the success of an AI program. Any problems with any of the steps may be magnified many times over when the final results are tallied.

Conception rates will vary depending on the type of semen, storage method, and AI technique used (Table 86-4).

In the event of program failure, the steps taken during the program should be carefully evaluated to find the cause of failure. The semen source and all steps in the collection, processing, freezing or shipping/storage of fresh as well as the thawing, handling, and actual insemination technique should be investigated. Insemination dose should be carefully calculated and semen evaluated at every step in the process that is practical.

Correct timing of insemination is critical. Every care should be taken to be as close as possible to the recommended time of insemination with the technique chosen. Ovulation time has been reported to vary between groups of ewes; hence the addition of GnRH may decrease variation in ovulation time and increase conception rates. Frozen semen is not viable for long in the reproductive tract, and it is important to time insemination carefully to approach 50 to 55 hours after progesterone removal.

The use of vasectomized rams or testosterone-treated wethers is recommended, as this will also help with ovulation synchrony. Some reports of vasectomized rams serving cervical or vaginally inseminated ewes have improved pregnancy rates (A.R. Cobb, personal communication). This also ensures that only those ewes in estrus are presented for insemination. Supplies and equipment available from several sources are listed in Box 86-1.

Box **86-1**

Equipment and Supplies

Electroejaculators
Lane Ram Ejaculator and
Pulsator III Electroejaculator
Lane Manufacturing, Inc.
2045 S. Valentia St.
Denver, CO 80231
(303) 745-2603
Bailey Ejaculator
Western Instruments
4950 York St.
Box 16428
Denver, CO 80216
(303) 295-7527

Transcervical Insemination Systems
The Guelph System for Transcervical AI
Gencor
Route 5
Guelph, Ontario, Canada
(519) 821-2150
Pipestone Veterinary Supply
Pipestone MN, 56164
(507) 825-3335
(800) 658-2525
sheep@pipevet.com
IMV
10 Rue Clemenceau, B.P. 76
61302 L'Aigle, France
IMV
6870 Shingle Creek Parkway
Suite 100
Minneapolis, MN 55430
(612) 560-4986
Minitube of America, Inc.
419 Venture Court
P.O. 930187
Verona, WI 53593
(800) 646-4882

Table **86-4**

Conception Rates Achieved in Well-Managed Programs Using Different Artificial Insemination (AI) Methods With Freshly Diluted or Frozen-Thawed Semen

Type of Semen	Dose of Progressively Motile Semen	Location of AI	Expected Lambing Rates (%)
Fresh	400×10^6	Vaginal	20–60
	200×10^6	Cervical	40–80
	20×10^6	Laparoscopic	70–100
	100×10^6	Transcervical	40–80
Frozen-thawed	400×10^6	Vaginal	5–20
	200×10^6	Cervical	
		Synchronized estrus	25–40
		Natural estrus	30–60
	20×10^6	Laparoscopic	40–80
	100×10^6	Transcervical	30–70

EMBRYO TRANSFER

Embryo transfer (ET) has had limited use in sheep because of its expense and invasive technique. Lack of access to the rectum and cervical anatomy contribute to the surgical, rather than nonsurgical, techniques being used for cattle and being developed in goats. The subsequent cost-benefit ratio has thus limited its use. In spite of this, ET has grown recently in popularity and is a useful technique in a variety of situations.

Benefits

ET enables increased genetic improvement through reduced generational interval, as ewe lambs may be used as soon as they reach puberty. These animals may have reduced birthing rates compared to more sexually mature donors, but if they have had several estrous cycles, the problem is not severe. Breeding strategies, such as multiple ovulation and ET schemes described by Smith (1988) is based on selection programs to create nucleus flocks of elite genetics and the use of multiple ETs to distribute these superior genetics at an accelerated rate.

Diseases are not readily spread by embryos and thus genetics can be successfully transferred nationally and internationally with regulatory restrictions the only limiting factor. Embryos can be processed to greatly reduce or eliminate the risk of disease transmission. ET can be used to repopulate flocks of known disease status, to move genes from country to country where certain diseases are endemic, and in disease research. Embryos can be collected while health data testing is being done.

Lastly, embryos may be frozen and stored indefinitely and shipped anywhere with reasonable expectation of pregnancy. New techniques such as one-step freezing and thawing reduce the time and equipment necessary to freeze embryos as well as reduce the difficulty in implantation. Genetic and disease evaluation programs can be instituted while the embryos are preserved.

Expected Success Rates

Success rates for sheep ET using fresh or frozen embryos are comparable with cattle and goat programs. Success of the program can be measured by the following:

1. Number of corpora lutea from superovulation (measures response)
2. Number of embryos obtained per corpora lutea (measures flushing success)
3. Number of fertilized embryos (measures breeding success)
4. Proportion of fertilized embryos suitable for fresh versus freezing techniques
5. Number of recipients that become pregnant (measure of embryo quality, recipient selection, management, and transfer technique)
6. Number of embryos transferred that produce lambs (measure of embryo quality, recipient management, selection, and technique)

Most authors average 8 to 12 embryos recovered per flush with 6 to 8 fertilized and 5 to 6 of these of transferable quality. In a well-managed program, two to four lambs should be obtained from each superovulation and transfer attempt. The range, however, is very wide, from 0 to 30 embryos and offspring per flush. Freezing of the embryos decreases the lambing rates by 10% to 20%. Embryo splitting raises the lambing rate to approximately 1:1 of the original transferable embryo number.

Factors Affecting Success

Recipient and donor selection will influence success rates. The more prolific breeds will usually respond better to superovulation and have higher transferable embryo rates than the less prolific breeds. Seasonal influences also play a role as late winter programs tend to have poorer flush results and maintenance of pregnancy becomes a problem. Ewe lambs also produce fewer embryos than more mature donors, but may make acceptable recipients, especially if they have gone through two or more estrous cycles. Recipients and donors must be in good body condition (BCS 3–3.5) and on a rising plane of nutrition. Fat ewes and those on an excessively high protein diet may have decreased embryonic survival rates.

Response to superovulation is the greatest single variable affecting success. Breed, season, BCS, drug used for superovulation, and dose and route of administration all affect the outcome. Timing of the program during the estrous cycle may also affect response. Programs using PMSG may have a prolonged period of estrogen production and delayed or incomplete ovulation. This estrogen production may also affect sperm transport through the reproductive tract and lead to decreased fertilization rates.

Better results are almost always obtained during the natural breeding season. It is also best to keep stress to a minimum to decrease premature luteal failure, which leads to decreased pregnancy rates.

Donors and recipients must be synchronized so that ovulation is no more than 24 hours apart. It is generally best for the recipient ewes to be behind rather than ahead of the donors if they cannot be same day.

Regulation of Embryo Export and Import

The potential for disease transmission by embryos is classified by the Office International des Ipizooties (OEI) and observed by the International Embryo Transfer Society (IETS). There are very few, if any, diseases that can be spread by properly processed and handled embryos. More research to further clarify disease spread is necessary. Most regulatory agencies have specific handling practices that must be followed and are detailed in the *Manual of the International Embryo Transfer Society* (Table 86-5).

Embryo Transfer Protocol

Almost all ET programs rely heavily on hormonal manipulation of the estrous cycle. Most, if not all, of the commonly used hormones are not commercially available in the United States. Most foreign countries have these hormones readily available for use, which puts the U. S. practitioner at a distinct disadvantage. Legislation is pending for use in “minor species” that may help with the supply of the drugs needed for such programs.

Table **86-5**

Disease Risk from Embryo Transfer

Category	Potential	Small Ruminant Disease
I	Sufficient evidence accrued to show risk is negligible provided that embryos are handled according to guidelines between collection and transfer	None
II	Substantial evidence accrued to show risk is negligible provided that embryos are handled correctly, and for which additional transfers are required to verify	None
III	Risk is negligible provided that embryos are properly handled, but for which additional in vitro and in vivo experimental data are required to verify existing data	Bluetongue Campylobacteriosis Foot and mouth Scrapie (sheep)
IV	Diseases for which preliminary research has begun	Ureaplasmosis Maedi-visna Pulmonary adenomatosis Scrapie (goats) Bluetongue (goats) *Brucella ovis* Border disease BSE agent

Superovulation of the Ewe

Follicle-stimulating hormone (FSH) products are usually derived from the ovine or porcine pituitary. The half-life of FSH is generally shorter than 6 hours and it may have varying levels of luteinizing hormone (LH). LH is associated with increased variability of response and reduced numbers of quality embryos. It is usually administered in a decreasing dosage regimen over a 3-day period starting 2 days before progestin withdrawal. It is generally administered twice a day with the final dose given 12 hours following progesterone withdrawal. The total dose of porcine FSH given is usually between 18 and 22 mg, depending on breed and weight. A sample program is outlined in Table 86-6. Other products are given in a similar manner, but whenever using an FSH product, careful attention to the product description and source must be given. There may be variation in product and LH level that may affect success of the program.

Equine chorionic gonadotropin (eCG), also referred to as pregnant mare serum gonadotropin (PMSG), has a longer half-life of approximately 72 hours. It also has more LH-like activity than FSH-like activity, and there is considerable variation between batches of the product. It is associated with overstimulation of the ovaries, hence produces large numbers of follicles and ovulations; but with residual, nonovulating follicles producing estrogen, a larger number of unfertilized ova (UFO) and poor-quality embryos may be produced. eCG, if used, is usually given 72 to 48 hours before progestin withdrawal at a dose of 1000 to 1500 IU, depending on breed and body size. The one advantage of eCG is that only a single injection is required.

A combination of FSH and eCG has been used in superovulation programs. A single, low dose of eCG (150–250 IU) given simultaneously with the first or second injection of FSH is reported to improve consistency of the program without the associated overstimulation associated with the larger dose of eCG by itself.

Table **86-6**

Example Superovulation and Synchronization Program

Day	Donor Procedure	Recipient Procedure
1	Begin progestin	Begin progestin
12	First FSH AM, second FSH PM	
13	Third FSH AM, fourth FSH PM	
14	Fifth FSH AM and progestin removed; sixth FSH PM	Remove progestin 500 IU PMSG
15	Ram introduced, GnRH 50 μg	Use teaser ram/50 μg GnRH
16	Ram remains or laparoscopic AI	
21	Remove food and water	Remove food and water
22	Embryo recovery (day 6–7 stage)	Transfer embryos

AI, artificial insemination; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone.

Breeding Management of Superovulated Ewes

Superovulation affects the dynamics of estrus, including time of onset and duration as well as sperm transport. All these factors affect fertilization rates and subsequent recovery of quality embryos. The optimal ram-to-ewe ratio for breeding superovulated ewes is 1:3 during the normal breeding season and 1:2 for out of season. The ram should not be allowed with the ewes until 36 hours

after progestin removal even though some will show estrus as soon as 18 to 24 hours after withdrawal.

As an alternative to natural mating, laparoscopic AI has been shown to increase fertilization rates. Recent work suggests that embryo recovery rates may be decreased in a laparoscopic AI program, but breeding 60 hours after progestin removal has been shown to maximize embryo fertilization and recovery rates. Administration of GnRH 30 or 36 hours after eCG or FSH respectively has been reported to improve the yield of fertilized ova from both naturally and laparoscopically bred ewes.

Anesthesia for Embryo Recovery and Transfer

Ewes may be induced with any of the common inhalant gas anesthetics or a combination of xylazine (0.2 mg/kg IM) followed in 5 to 10 minutes with ketamine (5 mg/kg IV or 5–11 mg/kg IM) with local anesthesia infiltrated at the surgical site. A lighter dose of either xylazine and a local or xylazine plus ketamine plus a local may be adequate for transfer of embryos by laparoscopic technique by a skilled operator. Xylazine may be reversed in 30 to 40 minutes with tolazoline (2–4 mg/kg slowly IV or IM) or yohimbine (0.125 mg/kg slowly IV or IM) after the procedure if desired by the operator. Both of these reversal drugs should be titrated to effect. The authors prefer the IM route because of the ease of administration and a decrease in cardiac effects.

Embryo Collection

Surgical collection of the embryos is a common procedure, and many highly skilled and trained operators do laparoscopic assisted recoveries. No technique yet exists for nonsurgical collection of embryos, as in other species, and because of the anatomy of the sheep cervix, a nonsurgical procedure may never be developed without special instrumentation. An experienced team may be able to flush as many as four ewes per hour. Supplies and equipment are readily available and the surgery is not technically difficult. Careful manipulation of the uterus is essential to minimize adhesions, and the tissues must be kept moist constantly with heparinized saline or lactated Ringer's solution. Multiple collections per season can be done, but it is recommended that after one to three recoveries the ewe be allowed to carry a pregnancy to term, as the enlarging uterus may help to break down any adhesions that may have formed. Most embryos are recovered 6 to 7 days after ovulation when the embryos are in the late morula or early blastocyst stage of development. At this time, the embryos are in the distal portion of the uterine horn. If the embryos are to be recovered earlier (usually day 3), they are recovered from the fallopian tubes by flushing through the fimbria into the uterus, where a catheter has been inserted near the uterotubal junction. This technique causes more adhesions of these delicate tissues and offers no advantages to the later flushing time.

The actual technique for surgical recovery consists of a ventral midline incision approximately 10 cm cranial to the mammary gland and just large enough for the surgeon to examine the ovaries and the uterus. The ovaries are first examined to determine response to the superovulation and an attempt to count number of corpora lutea made. The uterus is then exteriorized and carefully packed off using moist toweling and kept constantly moist. A stab incision is made at the base of the uterine horn with a mosquito forceps and a 10-gauge Foley catheter is inserted and the cuff inflated with 3 to 5 ml of air (Fig. 86-2). Another puncture is made near the uterotubal junction using a blunt 20-gauge needle and an open-ended tom-cat catheter is introduced and 25 to 40 ml of flushing medium are injected. The flushing medium is collected out of the Foley into a sterile container and any remaining medium is flushed from the uterus with air. The procedure is then repeated for the opposite horn.

Transfer of Embryos

Several different techniques are used to transfer embryos. The operator should be familiar with the equipment and skilled if using the laparoscopic method. Nonsurgical transfer has had some success using the Guelph system of transcervical insemination, but owing to the difficulties of consistently placing the embryos in the uterus, it cannot be recommended for routine use at this time.

Surgical transfer is performed through a midline laparotomy and the tip of the uterine horn exposed. A puncture is made into the lumen of the uterine horn with a blunt 20-gauge needle and a tom-cat catheter loaded with the embryos and a very small amount of medium injected into the uterus in the direction away from the oviduct. Most operators place two embryos at the one site and pregnancy rates are reported to be better than using either one or three embryos.

Laparoscopic transfer is widely used. It is faster and less invasive, but requires more skill. With this technique, the

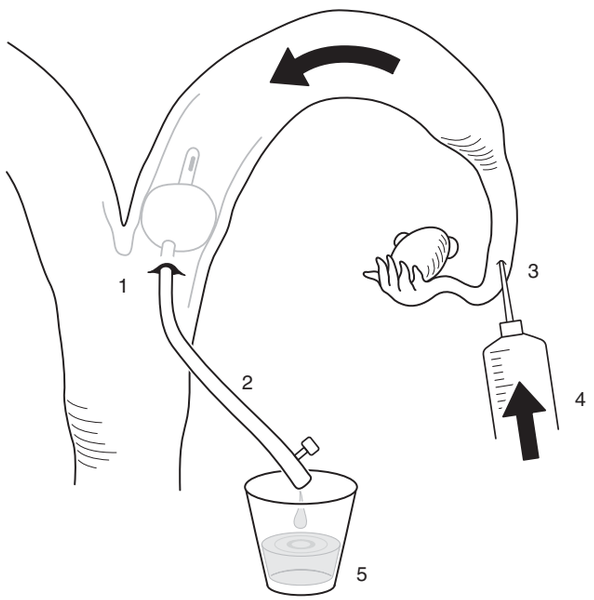

Fig. 86-2 Surgical recovery of embryos.

uterine horn ipsilateral to the corpus luteum (CL) is grasped and a blunt stab is made into the horn through the abdominal wall and a tomcat or similar catheter inserted into the uterus approximately 2 cm; the embryos in a small amount of medium are expelled into the uterus. A variation of this technique includes using the laparoscope to identify the CLs and using a grasping tool to then exteriorize the tip of the ipsilateral horn through the stab incision that is now lengthened just enough to allow catheterization and deposition of the embryos as described previously.

Embryo Bisection

Bisection of the embryo usually results in an increase in the number of offspring born to approximately 100% of the embryos transferred. An experienced technician can easily become skilled at bisecting embryos. Fresh bisected embryos can be frozen; however, frozen-thawed embryos are rarely bisected with high success rates. Use of bisection may be beneficial in some programs or in the production of identical twins for research purposes.

Assessing Embryo Quality

Accurate embryo grading is important to the success of an ET program. Poorer quality embryos should not be frozen and in some cases should not be transferred. They are graded and classified according to the guidelines of the IETS. Fertilization status, stage of development in relation to the day of recovery (normal rate of development versus retarded), and evidence of morphologic damage to the embryo and zona are all taken into account.

REPRODUCTIVE TECHNOLOGY IN THE FUTURE

The last 20 to 30 years have led to exciting discoveries in the knowledge of the reproductive cycle, ways to manipulate it, and the technology to use this knowledge to make advances in AI, ET, real-time ultrasound (RTU), in vitro fertilization (IVF), cloning, and so on. Future technologic advances will continue to be made and may bring many changes to sheep reproduction and the advancement of science and genetic progress. Some of these advancements, if proved successful in obtaining pregnancies, will be adopted in the sheep industry.

RTU has provided us with keys in the understanding of the estrous cycle of the ewe. It has allowed us to observe follicular dynamics, and hopefully, this knowledge will help to improve the efficacy of superovulation programs. A greater understanding of corpus luteum function and maintenance may help maintain pregnancies from transferred embryos. Supplementation of the CL with progestin or stimulating accessory CL formation may help maintain these embryos to establish into a viable pregnancy. Interferon-τ is the pregnancy recognition signal in the ewe, secreted by the developing embryo between 14 and 16 days of gestation. It stops prostaglandin F_2 alpha secretion, thus stopping CL regression. A recombinant interferon-τ now available theoretically may be given to relieve the compromised embryo from the burden of producing this signal and allow it time to recover.

Cryopreservation of embryos has progressed from the traditional three- or four-step procedures to the one-step procedure using ethylene glycol as the cryopreservative of choice. This has allowed direct thaw and transfer, which simplifies the use and handling of these embryos. Vitrification of the embryos also offers hope for higher pregnancy rates after thaw but at this time needs further development to bring it from the laboratory to the "field."

In Vitro Embryo Production

Of all the developments in reproductive technology over the past decade, the establishment of methods for in vitro embryo production (IVEP) may be the most significant in terms of its practical implications. The collection of immature oocytes from either living donors or ovaries collected at slaughter would eliminate the need for surgical collection of embryos from superovulated donors. The collection of mature oocytes from superovulated donors would eliminate the need for in vitro maturation of oocytes. Both adult ewes and juvenile lambs (8–12 weeks of age) have been successfully used to produce viable oocytes. In sheep IVEP, relatively high levels of maturation and fertilization (>60%) can be obtained in conjunction with good development rates to the blastocyst stage (>30%). Upon transfer, in vitro–derived sheep embryos give acceptable initial pregnancy rates (~50%); however, the majority of these fetuses are aborted during gestation, leaving only approximately 20% of transferred embryos to result in live lambs. A significant impediment to the widespread use of IVEP in sheep is the ram-to-ram variation in response of sperm to in vitro capacitation and culture. This is particularly pronounced with frozen-thawed semen. Despite setbacks, the IVEP procedure constitutes an enormous advancement in our ability to acquire large numbers of embryos by means other than from superovulated donors. The future should see the development of effective in vitro maturation, fertilization, and culture systems for all of the small ruminant species, resulting in embryos of high quality and viability.

Gamete Transfer

For all of the small ruminant species for which ET requires surgical procedures, the transfer of gametes (sperm and oocytes) may offer certain advantages over both traditional ET and IVEP techniques. Gamete transfer has been used extensively in the treatment of human infertility, where it has generally implied the transfer of a mixture of mature oocytes and processed sperm into the lumen of the donor's own uterine tube. Oocytes collected from a donor at slaughter or by laparoscopic aspiration would be matured and then transferred into the uterine tubes of a number of synchronized preovulatory recipients. At the time of donor oocyte transfer, all of the recipients' preovulatory follicles would be aspirated to remove the "native" oocytes, and sperm (fresh or frozen) would be

placed within the recipients' uterine lumen. Removal of native oocytes from the recipients' preovulatory follicles should lead to luteinization and the development of functional corpora lutea. The ability to acquire large numbers of oocytes from a single donor is considerably greater than the ability to recover an equivalent number of embryos over a restricted period of time. The principal advantage of gamete transfer over currently available IVEP procedures is that sperm capacitation, fertilization, and embryo development all occur in vivo, eliminating concerns regarding ram-to-ram variation and reduction in viability of cultured embryos. Developments in this area should include the evolution of oocyte transportation systems in which immature oocytes mature during transport, as well as the establishment of methods for the successful cryopreservation of oocytes. As more experience is gained in acquiring and maturing small ruminant oocytes, gamete transfer may become a routine procedure for transfer specialists of the future.

Embryo Multiplication

Techniques have been developed to multiply mammalian embryos and produce multiple live offspring from a single original embryo. Blastomeres from early cleavage-stage sheep embryos (two- and four-cell) can also be isolated and subsequently cultured to produce genetically identical embryos and offspring. Each of the individual blastomeres from a single four-cell sheep embryo maintains totipotency following isolation; thus, four identical offspring can be produced by the procedure. The combination of blastomere isolation and effective in vitro embryo production systems may lead to increased utilization of this technique in the future.

Further expansion of embryo multiplication has come with the development of cloning procedures, wherein blastomeres are fused with enucleated oocytes, which in turn reprogram the blastomere nucleus to develop as if it were a zygote. Cloning methods expand upon simple blastomere isolation procedures by permitting cells from later-stage embryos (eight- to 16-cell embryos, compact morulae, and inner cell mass cells from blastocysts) to be used as donor nuclei. Cloned embryos suffer the same insufficiencies that have plagued the use of in vitro derived and cultured sheep embryos in terms of fetal loss, increased size at birth, and postnatal mortality.

Beyond cloning with blastomeres, it has been proposed that permanent totipotent embryonic stem cell lines could be produced in sheep from which donor nuclei could be derived for cloning. Embryonic stem cell lines have been established from individual mouse embryos, which have been used to produce germ-line chimeras of altered genetic makeup. Embryonic stem lines have the potential to produce millions of identical totipotent cells, which may be studied or altered genetically before use in the production of embryos. The potential use of embryonic stem cell lines cannot currently be predicted in sheep, as they have not yet been produced in any small ruminant species, and it is not known whether their production will be biologically possible. Embryo multiplication methods may find a home in certain production situations wherein supply cannot meet demand and the value of offspring warrants the use of such techniques.

Marker-Assisted Selection

The use of genetic markers for the identification of animals carrying a specific gene is known as marker-assisted selection. Economically important major qualitative genes such as those for fecundity (Booroola-FecB; Thoka), health (scrapie resistance), growth and carcass characteristics (Callipyge), and sex have all been recently identified in sheep. The Booroola, Callipyge and male-specific genes have additionally been isolated after identification by molecular techniques. Genetic markers allow individuals to be screened for specific genes that may not be expressed (fecundity genes in males) or before phenotypic expression is normally observed in expressing animals. This advantage is most pronounced if marker-assisted selection is combined with ET and manipulation, and embryos can be screened for the presence of specific genes before they are transferred. Future developments should include the evolution of screening methods by which an embryo's genetic makeup could be determined through noninvasive methods. Marker-assisted selection will play an increasingly important role in livestock selection as more genes and gene markers are identified.

CONCLUSION

Practical programs for embryo production and transfer must be developed if veterinarians and producers are to take advantage of the promised gains in genetics and molecular biology. Efficient oocyte collection, consistently successful in vitro fertilization, rapid cryopreservation methods, and nonsurgical ET techniques are needed. Without an efficient method of animal propagation like ET, the expected advances in research will never be applied beyond the laboratory. Although the level of invasiveness has been greatly reduced by the introduction of laparoscopic methods, future development and increased application of embryo-based technologies will be closely tied to the development of nonsurgical procedures. Prospects for the development of widespread transcervical ET methods in sheep now appear promising, following the use of the transcervical insemination techniques to transfer embryos.

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CHAPTER 87

Clinical Reproductive Physiology of Ewes

NORMAN C. RAWLINGS and PAWEL M. BARTLEWSKI

REPRODUCTIVE PATTERNS

The ewe is seasonally polyestrous, with breeds derived from northern latitudes having a defined breeding season spanning the fall and winter months. Breeds developed closer to the Equator show less seasonality. The estrous cycle of the ewe is usually 16 to 17 days long, with estrus lasting approximately 30 to 36 hours. Spontaneous ovulation occurs late in estrus. Gestation length is approximately 147 days, with minor variations between breeds.[1]

REPRODUCTIVE CYCLE

Folliculogenesis

A resting pool of primordial follicles (containing primary oocytes) is established in the ewe prenatally, and in the adult ewe 3 to 4 follicles move from this pool and are committed to a growing pool (primary follicles) every day.[2] Although luteinizing hormone (LH) receptors are present in the theca and follicle-stimulating hormone (FSH) receptors are in granulosa cells quite early in follicular development (tertiary or preantral follicles of about 0.1 mm in diameter), folliculogenesis appears to be gonadotropin independent in sheep up to the 1- to 2-mm stage (early antral follicles). Most follicles are lost to atresia in a process of programmed cell death (apoptosis), particularly at the preantral and antral stages of development. Recent use of transrectal ultrasonography has allowed description of antral follicle dynamics (follicles with diameter > 2 mm) in the ewe (Fig. 87-1).[3] During the estrous cycle, antral follicles emerge or grow from the pool of follicles of 1 to 2 mm in diameter, approximately every 4 days (3–4 follicle waves per estrous cycle). The ovulatory follicle emerges around day 12 of the cycle. Unlike in cattle, the wave-like production of larger antral follicles in the ewe is not reflected in any change in the total numbers of small antral follicles (1–3 mm) in the ovary, apart from a small increase around ovulation.[3–5] In each wave, follicles reach a maximum diameter of 4 to 7 mm; maximum diameter is lower in prolific compared to nonprolific ewes. The largest, nonovulatory follicles of waves have a life-span of 5 to 12 days (emergence to regression or atresia), and life-span is longer for follicles emerging early in the cycle, before luteal progesterone secretion is fully established. Large antral follicle life-span in the ewe consists of a 2- to 4-day growing phase, a static phase (no apparent change in size, 1–4 days), and regressing phase (1–5 days). Each follicular wave emerges after the antral follicles of the previous wave have ceased active growth or are regressing in the ewe. However, in the ewe, there is little evidence for follicular dominance as seen in cattle, except perhaps in the ovulatory wave, where growth of the ovulatory follicle in the ewe exceeds that of other follicles in the wave.[4,6] Based on earlier, non-ultrasonographic studies, it was suggested that prolific ewes ovulated more follicles by reducing follicle atresia, recruiting more antral follicles into final growth and development, or by having a wider window of time over which ovulatory follicles were allowed to emerge.[2] In comparing nonprolific ewes to the prolific Finn, using ultrasonography, it was clear that the increased ovulation rate was due to the ovulation of more follicles from follicle waves emerging before the final wave of the cycle (extended period of recruitment).[3] Ovulation rate also increases from early to midbreeding season.[1]

In the cyclic ewe, the day of emergence of each follicle wave is synchronized to a small peak in serum concentrations of FSH (see Fig. 87-1).[3] Serum concentrations of estradiol increase from follicle wave emergence and peak when the largest follicle of a wave reaches its maximum size. Antral follicles of prolific breeds of sheep have fewer granulosa cells than those of nonprolific breeds, but steroidogenic potential is greater as serum concentrations of estradiol can be greater in prolific compared to nonprolific ewes throughout the cycle.[2] Termination of follicle growth and decreased secretion of estradiol could provide a signal for the subsequent peak in serum FSH concentrations that precedes and may induce the next wave of follicle emergence.[3,7] Although inhibin specifically regulates FSH secretion, it is probably less involved in regulating peaks in FSH secretion as inhibin is secreted from a wide size range of follicles. The duration and amplitude of peaks of FSH and estradiol do not vary across the estrous cycle.[3] LH pulse frequency is higher early in the cycle, before serum concentrations of progesterone have reached a maximum,[8] and this could explain the longer life-span of the first follicle wave of the cycle.[3] Interestingly, although mean serum FSH is greatest at wave emergence, FSH pulse frequency increases during the follicle growth phase of follicle waves.[7] In the ewe, growth of follicles beyond 2 mm in diameter is FSH dependent, but the larger follicles of waves can transfer their dependence from FSH to LH (LH receptors develop in granulosa cells).[2] This probably explains the ability of the preovulatory follicle to grow when serum FSH concentrations are at their lowest point in the cycle. The

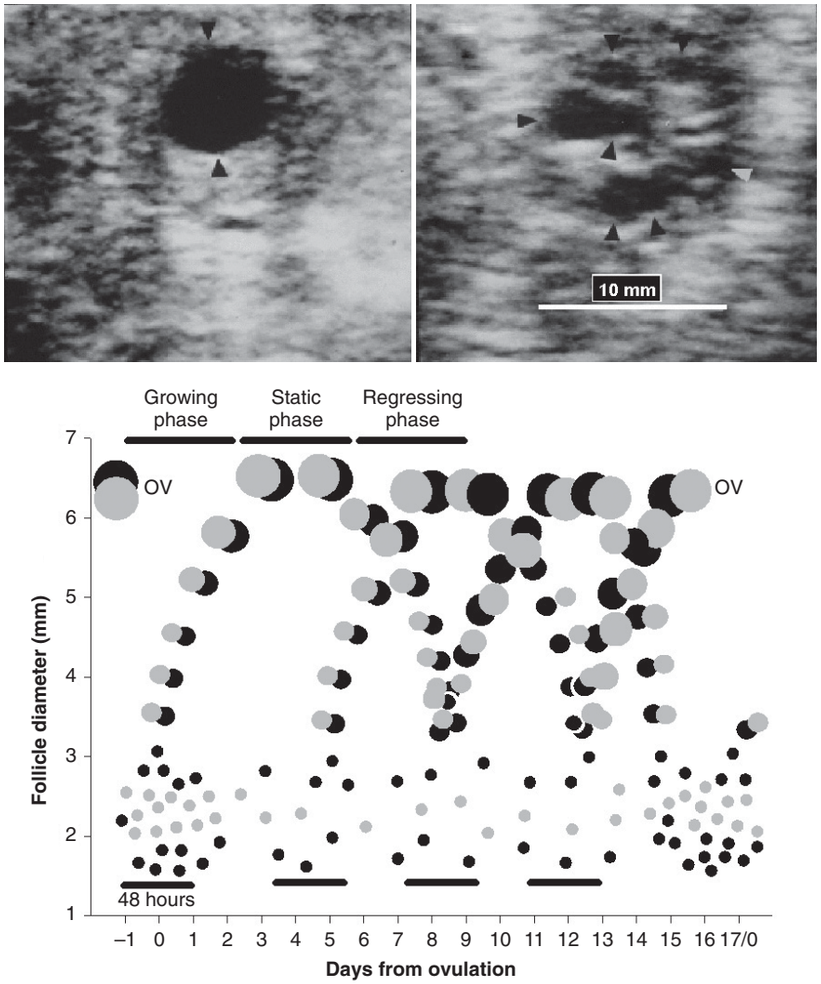

Fig. 87-1 The growth of ovarian antral follicles greater than 1 mm in diameter during the ewe's estrous cycle. The largest follicles (reaching ovulatory diameters of ≥5 mm) emerge in an orderly succession throughout the interovulatory period giving 3 to 4 follicular waves. Follicular emergence, or beginning of the growth of follicles to ovulatory sizes, occurs within a 48-hour period, approximately every 4 days during the luteal phase of the cycle. OV, ovulation. Photographic reproductions of ultrasonograms depict: *a*, a large antral follicle on the day before ovulation, and *b*, a cohort of small ovarian follicles (1–3 mm in diameter), obtained using transrectal ultrasonography of ovaries in Western white-faced ewes (nonprolific breed). Ultrasonography utilized a 7.5-MHz rectal probe connected to Aloka 500-SSD echo camera.

recent application of transrectal ovarian ultrasonography to the study of antral follicle dynamics in the ewe, described previously, promises to produce simple methods to increase ovulation rate and to synchronize follicle waves and ovulation to facilitate high fertility fixed-time insemination.[3]

In addition to endocrine regulation, follicular growth is subject to intraovarian control mechanisms. Insulin-like growth factors (IGFs) stimulate follicle growth and enhance gonadotropin responsiveness. The mRNA encoding IGF-II has been found in the theca of ovine follicles, but it is unclear if IGF-I is actually produced in the follicle or is supplied by production from the liver. Interestingly, a family of IGF binding proteins (IGFBPs) regulates the biologic activity of the IGFs, and a decrease in concentration of binding protein in antral follicles, as they grow, may enhance the biologic activity IGFs and gonadotropins. Inhibins and activins, members of the TGFβ superfamily, are produced by the growing follicle.[9] Inhibin has an α subunit coupled to either a βA or βB subunit; activins are dimers of βB, βA, or βB/βA. Activins promote granulosa cell proliferation and differentiation and estrogen production and are anti-atretogenic. Follistatin, an activin binding protein, can oppose the effects of activin. Inhibin α and inhibin/activin βA subunits are expressed in increasing amounts as antral follicles mature in the ewe; however, the local roles of inhibin are not as clear. Other potential regulators of follicle development are transforming growth factors α and β, epidermal growth factor (EGF) and fibroblast growth factor (FGF).

The antral follicle(s) emerging near the end of the ovine estrous cycle and destined to ovulate does not necessarily grow any larger than nonovulatory follicles of earlier waves, nor does it produce more estrogen.[3] The ovulatory follicle undergoes final development largely dependent on LH, and the preovulatory LH surge causes ovulation, luteinization, and maturation of the primary oocyte to a secondary oocyte.[2] Recent work in the ewe has shown that the preovulatory LH signal causes secretion of plasminogen activator from the ovarian and ovulatory follicle wall.[10] Plasmin activates collegenases, which disrupt the ovarian and follicle wall; plasmin also causes release of tumor necrosis factor alpha (TNF_α), an apoptotic agent.

Luteal Function

The collapsed, ovulated follicle contains a blood clot and little luteal tissue. This structure grows rapidly and organizes; it is called a corpus hemorrhagicum until day 4 or 5 of the cycle, when it starts to produce significant amounts of progesterone, and thereafter it is called a corpus luteum. In Western white-faced ewes, serum progesterone concentrations increase to around day 12 of the cycle and then decline to day 16; however, from days 8 to 13 serum progesterone concentrations do not differ significantly (see Fig. 87-1). Based on recent ultrasonographic data from Western white-faced ewes,[11] luteal volume increased to day 10 after estrus and then declined to day 17 of the cycle; however, luteal volumes did not differ significantly between days 8 and 13. Therefore, in Western white-faced ewes, a rapid increase in serum concentrations of progesterone preceded a marked increase in luteal volume. At luteolysis, functional and structural luteal regression seemed to commence coincidentally but the decline in serum concentrations of progesterone was somewhat sharper than for luteal volume. Prolific Finn ewes produced more but smaller corpora lutea and had lower serum concentrations of progesterone compared to Western white-faced ewes.[11] Interestingly, ultrasonographic studies in the ewe have revealed luteinized follicles and short-lived corpora lutea developing in parallel with normal corpora lutea.[3,7,11] In two ewes with the estrus cycle of 23 days each, normal life-span corpora lutea appeared to almost completely regress and then redevelop for a further luteal phase. In addition, serum progesterone concentrations vary with season; concentrations are greater in midbreeding season compared to the early or late season.[11]

The corpus luteum consists of small luteal cells derived from the theca cells of the follicle, large luteal cells derived from granulosa cells, fibroblasts, and endothelial cells. Only small luteal cells are LH responsive but the large cells probably produce most of the progesterone; prostaglandin receptors are restricted to large luteal cells. During luteogenesis small luteal cells divide but large luteal cells increase in size. Pulsed LH secretion does not appear to be necessary to maintain luteal progesterone secretion in the ewe but maintenance of progesterone secretion and luteal weights requires basal LH secretion.

Luteolysis is caused by pulses of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the endometrium of the uterine horn ipsilateral to the corpus luteum, between days 14 and 17 of the estrous cycle.[12] The $PGF_{2\alpha}$ is transported by a countercurrent exchange between the uterine vein and ovarian artery and the lymphatics are also involved. The pulsed release of $PGF_{2\alpha}$ is driven by pulsed secretion of oxytocin from the corpus luteum and perhaps the posterior pituitary. What actually initiates the pulsed secretion of oxytocin and $PGF_{2\alpha}$ is unclear. For oxytocin to stimulate $PGF_{2\alpha}$ secretion, the development of endometrial oxytocin receptors is pivotal. Development of oxytocin receptors is blocked until days 10 to 12 of the cycle by progesterone. However, progesterone eventually downregulates its own receptors, allowing an increase in estradiol receptors; estradiol is then able to stimulate production of oxytocin receptors.[12] Prostaglandin $F_{2\alpha}$ can cause functional luteolysis (loss of progesterone secretory ability) through receptors on large luteal cells and by disrupting cholesterol transport across mitochondrial membranes.[12] $PGF_{2\alpha}$ can also cause the release of endothelin 1 from endothelial cells in the corpus luteum. Endothelin 1 reduces progesterone secretion from luteal cells as well as causing vasoconstriction. Endothelin 1 may also attract monocytes to the corpus luteum and the release of transforming growth factor α from macrophages could be an early signal for apoptosis and structural regression of the corpus luteum. Prostaglandins (10–15 mg $PGF_{2\alpha}$ IM) have been used to cause luteolysis and synchronize estrus in ewes; the corpus luteum is only responsive from day 4 of the cycle.[1] Estrus occurs about 40 hours after $PGF_{2\alpha}$ but the estrous response and fertility to breeding with $PGF_{2\alpha}$ treatment are more variable than with other methods of estrous synchronization.

Endocrine Regulation

Gonadotropin-releasing hormone (GnRH), and hence LH and FSH, are released in a pulsatile manner in the ewe.[8] In blood sampling sites distant from the pituitary effluent, the pulsatile pattern of serum concentrations of FSH is unclear, possibly due to its long half-life. During the luteal phase of the ovine estrous cycle, LH and FSH secretion is suppressed and LH pulse frequency is low (1 to 2 pulses per 6 hours; see Fig. 87-1). LH pulse frequency is decreased by the negative feedback effects of progesterone and estradiol, operating in concert.[8,13] At luteolysis as progesterone concentrations decrease, LH pulse frequency increases (1 to 2 pulses per hour). Increased LH pulse frequency increases estradiol production from the follicles destined to ovulate, and rising titers of estradiol in the absence of progesterone elicit a surge of LH/FSH secretion lasting 12 to 14 hours.[8,14] Although LH/GnRH pulsatility persists throughout the preovulatory surge, a surge of GnRH is also released. Ovulation occurs about 24 hours after the onset of the preovulatory LH surge and the surge occurs early in estrus. Following the preovulatory LH surge, elevated LH pulse frequency is initially maintained, suppressed eventually by the increasing progesterone secretion from the corpus hemorrhagicum/luteum.[14] Following ovulation, a second peak in serum FSH concentration is seen, heralding the first wave of antral follicle growth of the subsequent estrous cycle.[3] Treatment with exogenous progesterone suppresses LH pulse frequency, and progesterone or synthetic progestogens in Silastic rubber intravaginal devices or sponges, respectively, will suppress cyclicity and give good estrous synchrony when withdrawn.[1,13] Intravaginal devices or sponges are left in place for 12 to 14 days, and ewes come into estrus about 48 hours after sponge removal.

Estrous Behavior

The ewe in estrus will seek out the ram and stand as he conducts his investigative courtship. The estrous ewe will often shake her tail and turn her head to look back at the ram. Ewes out of estrus will squat and urinate at the ram's approach and move away if he approaches her

aggressively. Vulval swelling is often seen in the estrous ewe with a copious thin vaginal mucous secretion. Estral vaginal mucus dries into a typical fern crystal pattern on a glass slide.[15] Estrus is clearly timed by a period of progesterone priming (luteal phase), following by progesterone withdrawal and exposure to estradiol. Timing of estrus and the preovulatory LH surge are influenced by the peak concentrations of progesterone during the luteal phase and the slope of the progesterone decline at luteolysis;[16] higher luteal progesterone concentrations and a slower decline delay estrus and the LH surge.

MATERNAL RECOGNITION OF PREGNANCY

Progesterone is essential for the maintenance of pregnancy and following conception the corpus luteum of the cycle has to be maintained. The conceptus must be present by day 12 to 13 of the cycle in the ewe to rescue the corpus luteum from luteolysis. The ovine trophectoderm produces interferon tau (IFN-τ) between days 10 and 21 of pregnancy, with a marked increase in production between days 13 and 15.[12] Interferon tau blocks the pulsatile secretion of $PGF_{2\alpha}$, but not basal secretion, probably by suppressing production of the endometrial estrogen receptor and hence the increase in oxytocin receptor numbers required to trigger the luteolytic release of $PGF_{2\alpha}$. Embryo elongation is important to provide an antiluteolytic signal to the uterine horn ipsilateral to the corpus luteum, and ewes with higher serum concentrations of progesterone very early in the cycle (days 2–4) develop faster and produce more IFN-τ.[17] Therefore, there may be a role for progesterone supplementation in early pregnancy to reduce the chance of early embryonic death.

PREGNANCY AND PARTURITION

The corpus luteum is essential to pregnancy until day 50 or 60 of gestation; thereafter, the placenta assumes the role of progesterone production.[18] Serum concentrations of progesterone increase markedly after day 80 of gestation.

Pregnancy Diagnosis

Real-time ultrasonography can be used to detect pregnancy and assess fetal numbers as early as day 25 of gestation. Pregnancy-specific protein B (PSPB) can be detected as early as day 20 of gestation. Progesterone concentrations in serum in late gestation differ between ewes carrying singletons, twins, and triplets as the placental mass varies with fetal numbers. However, there is too much overlap between ewes with different fetal numbers for this to be useful in predicting fetal numbers.

Parturition

Apart from isolated, uncoordinated contractions, the uterus of the ewe remains quiescent until the forceful and coordinated parturient uterine activity that lasts just 9 to 12 hours (Fig. 87-2).[19] Once initiated, uterine contractions increase in amplitude and frequency, in coordinated waves, from ovarian to cervical ends. During this period, the cervix dilates under the mechanical influence of uterine contraction and fetal pressure. Fetal expulsion occupies no more than the final 25% of the period of parturient uterine activity, and this is usually followed by placental expulsion within several hours. Over the last days to weeks before parturition, connective tissue in the cervix and pelvic ligaments relaxes, and in preparation for delivery, the fetus is orientated into the birth position.

Parturition in the ewe is initiated by the fetus.[18] During the last 2 to 3 weeks of gestation secretion of corticotropic releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and adrenal cortisol and androstenedione increase in the fetus; serum concentrations of cortisol increase rapidly in the fetus over the last 2 to 3 days before delivery (see Fig. 87-2). It is not clear whether this increase in activity in the fetal hypothalamic-pituitary-

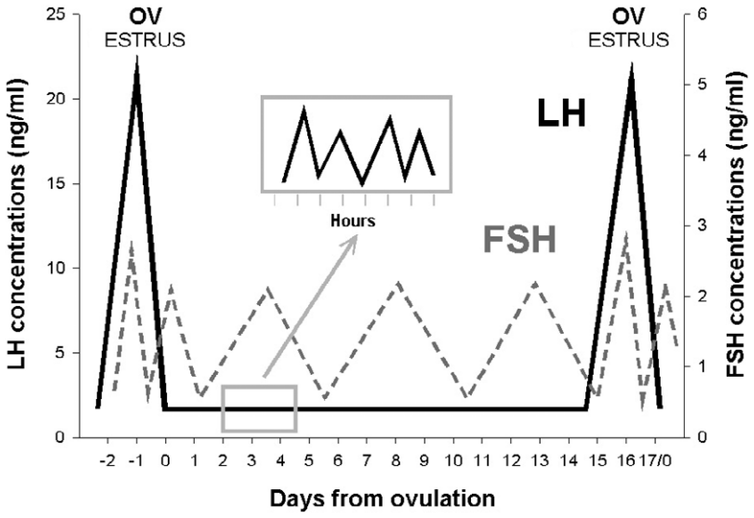

Fig. 87-2 Diagram illustrating changes in circulating concentrations of gonadotropic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), at different stages of the ovine estrous cycle.

adrenal axis is purely a developmental progression or an increased sensitivity to stressors such as hypoxia.[21] In addition, increased estrogen concentrations in the fetus (placental origin) increase ACTH secretion and androstenedione and prostaglandin E_2 (placental origin) can reduce the negative feedback regulation of ACTH secretion by cortisol (Figs. 87-3 and 87-4). During the last 2 to 3 weeks of gestation, the fetal adrenal gland grows rapidly and increases in sensitivity to ACTH.[18,22] Increased fetal cortisol concentrations primarily enhance placental 17α-hydroxylase activity to force a change from placental progesterone production to estrogen production.[20,22] In the ewe, serum concentrations of progesterone decline over the last 5 to 15 days of gestation while serum estradiol concentrations gradually increase; however, the major and most dramatic increase in estradiol is restricted to the last 1 to 2 days before fetal delivery.[18,19] The shift in the estrogen-to-progesterone ratio is critical for initiating parturition. The decline in progesterone secretion and increase in estrogen production promote the production of myometrial contractile proteins, enzymes needed for energy production, and gives a marked increase in sensitivity to oxytocin (increased receptor numbers). Progesterone tends to have a hyperpolarizing effect on the myometrium while estrogens tend to render the myometrium more hypopolarized or more unstable and responsive to stimulation. These effects involve influences on production of ion channels and calcium handling in the myometrium. The shift in the estrogen-to-progesterone ratio increases gap junction production in the uterus, enhancing electrophysiologic coupling. The initiation of parturition and development of contractibility involves stimulation by $PGF_{2\alpha}$, whose production by the placenta is enhanced by the shift from progesterone to estrogen production. This same steroidogenic shift enhances posterior pituitary production of pulsed secretion of oxytocin, which stimulates uterine contraction both directly and by release of prostaglandins

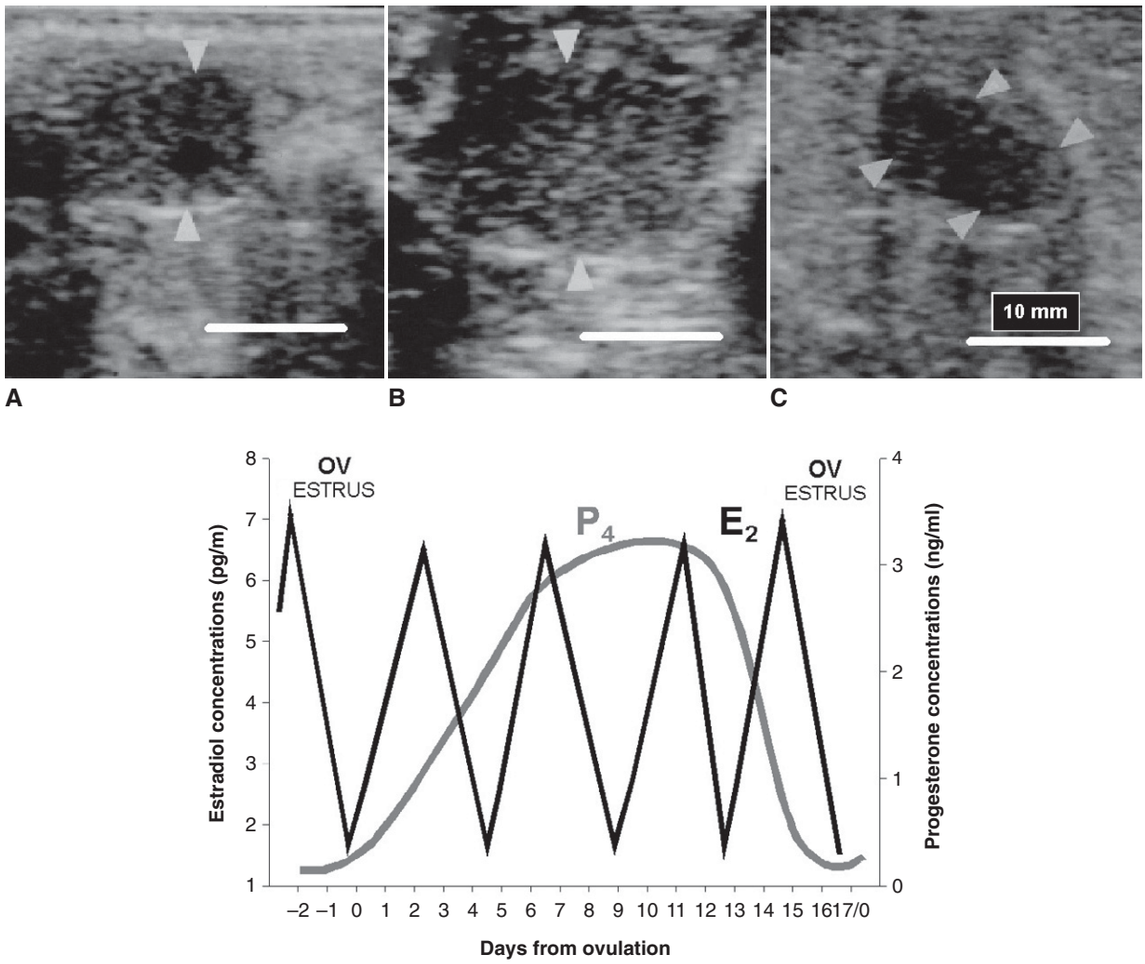

Fig. 87-3 Changes in mean serum concentrations of progesterone (P_4) and estradiol 17-β (E_2) throughout the sheep estrous cycle. Secretion of luteal P_4 closely reflects physical changes in corpora lutea (CL). Peaks of transient increases in serum E_2 concentrations coincide with the end of the growth phase of the largest follicles of waves, and follicular wave emergence is associated with the onset of successive E_2 fluctuations. Photographic reproductions of ultrasonograms depict: *A*, corpus luteum (CL) containing a small central cavity in the ovary of a ewe on day 5 of the estrous cycle; *B*, ovine CL detected at midcycle (day 11); and *C*, two regressing CL detected on day 15 after ovulation.

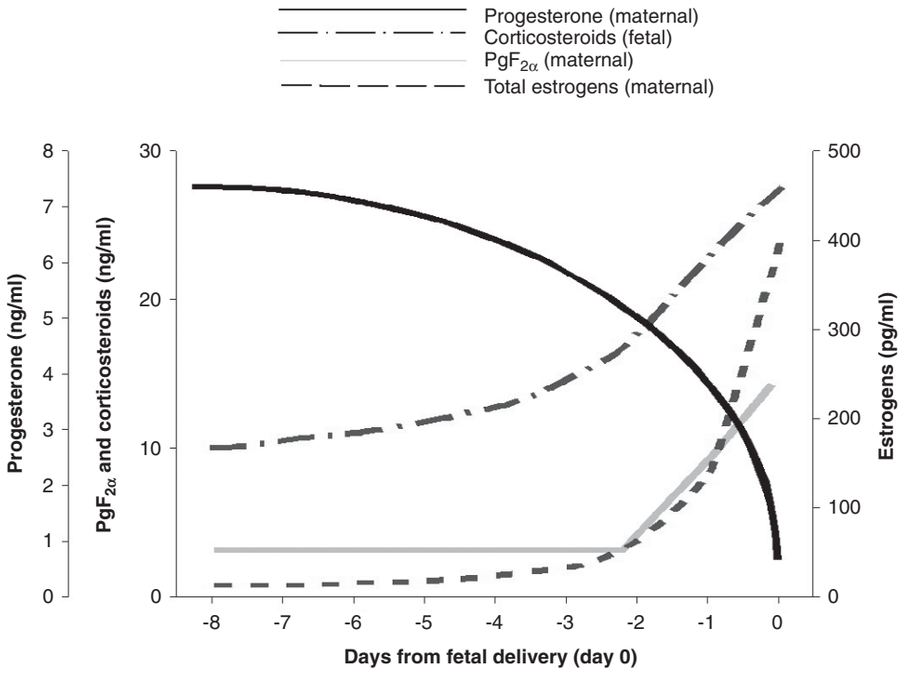

Fig. 87-4 Endocrine changes occurring before and during parturition in the ovine fetus and pregnant ewe.

from the endometrium. As the fetal head enters the cervix and contacts the vaginal wall, there is a neuroendocrine release of oxytocin, and spinal reflexes bring abdominal contractions to bear to reinforce each uterine contraction. The relaxation of pelvic ligaments and cervical softening (ripening) are due to release of collagenase under the influence of estrogens and PGE_2; PGE_2 is produced in increasing amounts by the placenta and cervical mucosa over the last 2 to 3 weeks of gestation. The hormone relaxin is probably also involved in the process of parturition in the ewe, inhibiting myometrial contractility until near term and causing cervical ripening.

Induction of Parturition

Parturition can be induced with synthetic glucocorticoids (15–20 mg dexamethasone IM) given within 4 to 5 days of expected term.[1] Induction is successful earlier in gestation (20–30 days before term), but fetal dysmaturity is a problem. The interval from induction to lambing averages about 48 hours. Estrogens can also be used (estradiol benzoate 2–20 mg IM), with an interval from induction to lambing of around 40 to 45 hours.[23] It is obviously necessary to have accurate breeding dates to avoid premature induction of lambing; however, if a low dose of estradiol benzoate is used (2 mg IM), ewes 17 days preterm are not induced to lamb. Prostaglandins will terminate pregnancy up to about day 30 of gestation but are not useful thereafter, probably because they induce luteolysis and later in gestation the ewe produces progesterone from the placenta.[1]

POSTPARTUM PERIOD

Ovarian follicular development is suppressed during pregnancy and until days 20 to 30 post partum, particularly in the ovaries containing or previously containing corpora lutea.[24] This suppression appears to be due to, or a residual effect of, the fetus. In general, postpartum recovery of ovarian function is not a practical problem as ewes generally lamb into their seasonal anestrus.

SEASONALITY

Ewes derived from northern latitudes and managed in temperate climates are regarded as short-day breeders. The ewe appears to have an endogenous rhythm to its seasonality, with a periodicity of 1 year. This endogenous rhythm is cued to season by photoperiodic signals. This endogenous annual cycle is maintained when ewes are exposed to constant day lengths but becomes disassociated from season.[8] Photoperiod influences reproduction through the pattern of pineal melatonin secretion. Melatonin is secreted during the hours of darkness and hence the period of melatonin secretion during each 24-hour period varies with day (night) length. Information from the eye is transmitted to the suprachiasmatic nucleus of the hypothalamus, and from there it is routed to thoracic spinal nerves and by way of the superior cervical ganglion to the pineal gland. Not all the annual melatonin secretory profile is used by the ewe to cue its annual reproductive rhythm to season. Spring and fall components may have an influence but the summer pattern of melatonin secretion (summer solstice to autumn equinox) is most critical. The sites where melatonin interfaces to regulate reproductive seasonality is unclear, but it is probably in the medial basal hypothalamus in sheep.

The onset of seasonal anestrus is abrupt in sheep, with the failure to ovulate at the end of a luteal phase. The growth of waves of antral follicles continues during anestrus.[25–27] Some irregularity in waves is noted transiently at the end of the breeding season but not at the onset of the next season. Waves are initiated by peaks in

FSH secretion and follicles grow to ovulatory sizes; however, estrogen production is limited in anestrus. Wave periodicity is around 5 days. The number of small follicles (<3 mm) is greater in anestrus than the breeding season. At the onset of the breeding season there is a variable length period of progesterone secretion (normal luteal phase length and shorter) that is produced by a preovulatory-like LH surge, but is not preceded by ovulation or estrus. The source of this progesterone is unclear; in some ewes it emanates from luteinized follicles. The second LH surge of the season is accompanied by ovulation and estrus. At the onset of anestrus, specific dopaminergic neurons are activated that inhibit GnRH pulse frequency. These neurons are sensitive to estradiol, such that estradiol has a powerful inhibition of LH pulse frequency during anestrus.[8] These inhibitory influences are reversed at the onset of the following breeding season and melatonin secretory patterns are able to cue these shifts in inhibitory influences. Interestingly, an intact thyroid is required for the onset of anestrus.[8]

Estrus can be induced in anestrus ewes by a 12-day treatment with a progestagen-impregnated intravaginal sponge; 500 IU of equine chorionic gonadotropin (eCG) is given at sponge removal. Without eCG, few ewes show estrus and ovulate. The success of synchronization in anestrus depends on the breed used and stage of anestrus. Fertility is better close to the breeding season compared to deep anestrus and ewes with a shorter anestrus respond more consistently. Introduction of a novel ram to anestrus ewes, after a period of isolation from the male, will induce increased pulsed secretion of LH within minutes, an LH surge within 48 hours, followed by a silent ovulation. The following luteal phase can be normal length or short (6–7 days) but is followed by estrus and ovulation. The short luteal phase can be prevented by exposure to progesterone before ram introduction. Again, the response to the ram varies and works best in ewes late in anestrus. Artificial day-length manipulation can be used to advance the breeding season. A typical treatment would be a month of exposure to long days in late winter, spring, or early summer, followed by an abrupt or gradual reduction to short days. Response time varies depending on the season in which the treatment is started, being shorter the closer treatment is applied to the onset of the breeding season. Ewes have also been treated with melatonin orally or by implant to advance the breeding season; treatment will not prolong the season. Treatments given in May or June, for 60 to 70 days, have been effective in advancing the breeding season and improving fertility early in the season but results have not been positive in all cases.

SEXUAL MATURATION IN THE EWE LAMB

Suffolk and Western white-faced ewe lambs show first pubertal estrus at around 30 weeks of age.[28,29] The onset of cyclicity at sexual maturity is similar to the adult ewe in transition from anestrus to the breeding season, in that silent ovulation or a short luteal phase may be seen. In the ewe lamb, LH pulse frequency increases markedly several weeks before puberty. Using ultrasonography, numbers of large antral follicles and maximum follicle size were shown to increase from 8 to 24 weeks of age, with a peak at 16 weeks of age.[28,29] Timing of sexual maturation in ewes is dependent on nutrition and an adequate rate of growth as well as photoperiodic influences. The well-grown ewe lamb must experience long days and then decreasing day length to show first estrus and the breeding season. Lambs need to be born early enough in the year to be able to grow adequately to show estrus in the fall. Again, as in the adult transitioning out of anestrus, LH pulse frequency increases owing to a decrease in responsiveness to estradiol negative feedback. This decrease in negative feedback is influenced and cued by adequate growth and photoperiod.

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CHAPTER 88

Sheep Breeding Strategies

DUANE H. KEISLER

Sheep are among the most versatile of the livestock species managed today in adaptability to diverse production environments and purveyors of various products throughout the world. Certainly the origin of this diversity evolves from the 400 to 800 different breeds of sheep that exist worldwide. As an example, although most sheep breeds birth 1 to 3 lambs per lambing, unique breeds such as the Booroola Merino exists functionally as litter-bearing ruminants, giving birth to 6 or more offspring per lambing. Compounding this diversity among breeds is the fact that sheep are seasonal short-day breeders, and adherence to or deviation from the short-day breeding activity will vary with the geographic latitude of their origin and subsequent management. Therefore, as advantageous as this diversity may be, it also imposes the liability that one set of recommendations cannot fit all paradigms. The objective with this chapter therefore is to describe the major variables that influence breeding activity and subsequent lambing performance in a manner that allows the informed individual to plan or diagnose schemes for managing breeding strategies that optimize ewe production performance.

CONSIDERATIONS IN SELECTING A BREEDING STRATEGY

Breeding Ewe Lambs

From a producer's perspective, a major dilemma exists in the decision of which ewe lambs should be kept as replacement females to enter the flock and which ewe lambs should be sold to capture market lamb prices while that "window of opportunity" still exists. Certainly the animal's genetic merit, production potential, and structural integrity are important variables to consider, but perhaps not as critical, but still significant, are the answers to the following two questions: (1) Will the selected ewe lambs ever achieve reproductive competence—(given that 10% to 20% of ewe lambs may possess some anomaly that prohibits the ability to ever reproduce)? (2) If the ewe lambs are capable of reproducing, when will they exhibit that capability (research has noted that ewe lambs that reach puberty at an early age are more reproductively efficient throughout their lifetime than are ewe lambs that reach puberty at an older

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age)? The factors that regulate the age at which lambs reach puberty can be categorized as genetic, environmental, and the interactive effects of genetics and environment.

Lambs of the British breeds, such as Southdown, Shropshire, Oxford, Hampshire, Suffolk, and Dorset, generally reach puberty at an earlier age than do lambs of the Spanish breeds, such as Merino, Rambouillet, Columbia, and Corriedale. In general, most lambs reach puberty within 6 to 10 months of birth, providing that environmental determinants such as nutrient availability, health status, and photoperiodic environment are favorable. Prolific breeds, such as Romanov and Finnish Landrace, may reach puberty at earlier ages. Therefore, among the first considerations to be made in assessing a realistic breeding scheme is whether the ewe lambs in question are of *sufficient age to reproduce*. Once some reasonable determination has been made that the lambs meet or exceed the breed-defined age parameters to reproduce, focus can then be placed on environmental conditions that permit the lamb to express its minimum age at puberty.

There are many environmental determinants of a lamb's age at puberty; however, the most frequently overlooked factor is the nutritional status of the developing lamb. The relationship between age and weight (or "condition") of lambs at puberty is critical. Figure 88-1 illustrates this relationship for lambs growing at three different rates. The long-dash line illustrates the growth rate of lambs belonging to a producer who overfeeds his lambs in an effort to minimize their age at puberty. What this producer will find is that the "long-dash line" lambs do not reach puberty until they also satisfy the minimum age requirements. The cost of this mismanagement or unnecessary investment in excess feed is evident in the excess weight or condition carried by the "long-dash line" lambs as they start to cycle. Furthermore, evidence now exists that animals carrying excess fat have reduced fertility.

In stark contrast to the overconditioned lambs represented by the long-dash line are the lambs managed by a producer who inadequately feeds his lambs (represented by the short-dash line), maintaining the presumption that the "short-dash line" lambs will begin to reproduce when they are of sufficient age. Clearly, in this situation the "short-dash line" lambs will surpass their genetically defined minimum age at puberty owing to insufficient metabolic reserves to initiate the reproductive process. In the ideal situation (represented by the solid line), a producer should feed lambs to a target weight and age to conserve feed without sacrificing reproductive performance. The minimum age requirement is dictated by the breed and the minimum weight requirement is generally 68% of their projected adult body weight. Producers who choose to breed ewe lambs at the minimum limits of their age and weight should manage the lambs so that they satisfy both minimum requirements at least 2 to 3 weeks prior to the time it is desired to have them cycling.

When ewe lambs start to cycle, the next logical question is when should they *first be bred*. Much research has provided evidence to support the argument that with few exceptions ewes should be selected, managed, and bred to produce offspring by 1 year of age. The benefits are reduced preproduction costs, shortened generation intervals (thus permitting more rapid genetic progress), and finally, increased lifetime productivity. The risks are slowed growth rates of the dam, low initial conception rates, and potential birthing or mothering problems as the result of the young dams giving birth to multiple or oversized offspring. The key to making the correct decision lies in the ability to plan and manage this process. For example, the earlier lambs are bred in the breeding season, the greater the probability those young dams will conceive single offspring, thus reducing the incidence of birthing difficulties associated with multiple births and reducing the risk of insufficient milk for multiple offspring. Furthermore, lambs could be bred to breeds of rams noted for their lambing ease to minimize potential lambing difficulty. Perhaps most the valuable information gained in efforts to breed ewe lambs at a young age is the identification and thus ability to select ewe lambs with the ability to reproduce.

Nonetheless, once the young dams lamb, it is critically important to feed them adequately to ensure their con-

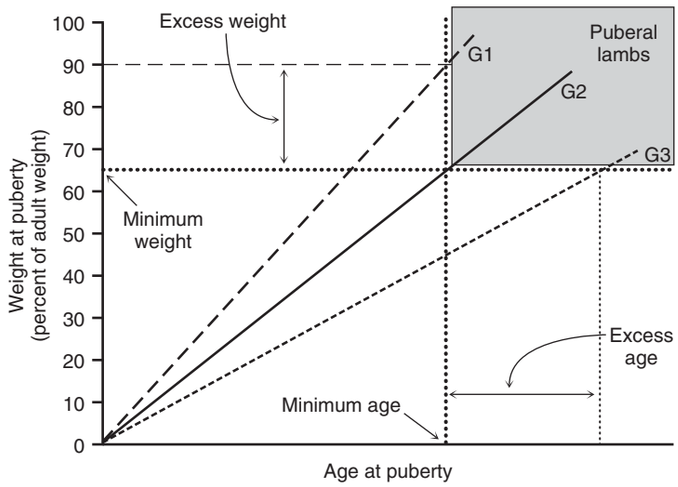

Fig. 88-1 Relationship between age and weight of lambs at puberty that are growing at three different rates.

tinued growth and development while lactating and beyond. Failure to adequately feed dams that lamb at a young age is one of the most common mistakes made by producers.

Also important in selecting replacement ewe lambs is their date of birth (Fig. 88-2). The reason for this selection variable is because in order for ewe lambs to reach puberty, they must first be exposed at an early age to increasing day lengths (summer solstice = June 21) and later to decreasing day lengths (winter solstice = Dec. 21), the latter of which provides the stimulus for them to begin to cycle. Offspring born prior to June have the greatest likelihood of reaching puberty and starting to cycle during the subsequent fall or winter if conditions are favorable, whereas offspring born after June are less likely to reach puberty during the first fall or winter but will wait until the following fall or winter to reach puberty at over 1 year of age.

Finally, it should be noted (as illustrated in Fig. 88-3) that for ewe lambs, the breeding season will typically begin 3 weeks after the adult ewes and cease 3 weeks prior to the adult ewes. The consequence of these restrictions is reduced opportunity to conceive at a time in the ewe's life when conception rates are typically low to begin with—thus the concern over delayed age at puberty.

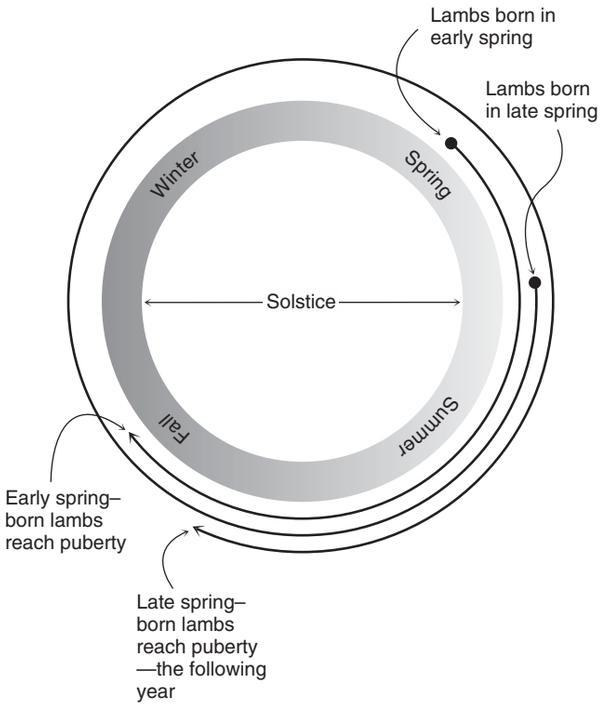

Fig. 88-2 Relationship between date of lamb's birth, length of daylight, and age at puberty.

Breeding Adult Ewes

A proportion of all ewes will cycle year round (also represented in Fig. 88-3 and Table 88-1) and typically for ewes living closer to the Equator (e.g., Idaho versus Texas), the proportion of ewes cycling year round increases. In contrast, it should be noted that relocation of breeds of sheep that are adapted to the equatorial latitude to locales more distant from the equator will result in a lower proportion of ewes cycling year round. As an example, efforts to establish a flock of ewes that breed year round in New York simply by importing ewes from the tropics will not be effective.

As an added note of caution, behavioral estrus and the occurrence of ovulation are not necessarily co-dependent. With this in mind, it is important to note that the proportion of ewes ovulating in Table 88-1 is often greater than the proportion of ewes that display estrus. This phenomenon is especially true during the times of the year when ewes make the transition into or out of the breeding season. The reason for this disconnect between estrus and ovulation may be due to either a failure in the ability to detect estrus or failure by the ewe to show estrus at the time of ovulation (silent estrus).

Finally, among ewes that cycle throughout the year, ovulation rate will vary with season of the year (see Table

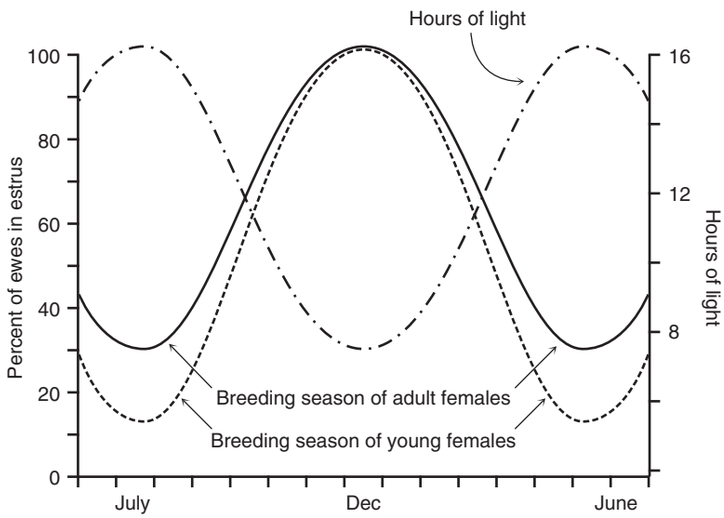

Fig. 88-3 Breeding activity in sheep in the Northern Hemisphere relative to length of day and month of the year. The solid line symbolizes ewes with longer breeding seasons than those represented by the short dashed line.

Table **88-1**

Estrus, Ovulation, and Ovulation Rate in Ewes in Idaho and Texas

	Ewes in Estrus (%)		Ewes Ovulating (%)		Ovulation Rate
	Idaho	*Texas*	*Idaho*	*Texas*	*Idaho*
JAN	100	100	100	100	1.89
FEB	100	100	100	94	1.57
MAR	89	40	94	52	1.50
APR	26	38	32	32	1.37
MAY	2	31	2	31	1.00
JUN	7	44	7	75	1.00
JLY	6	94	6	94	1.00
AUG	12	86	41	100	1.75
SEP	88	94	100	94	1.72
OCT	100	94	94	100	1.80
NOV	100	97	100	91	1.86
DEC	100	100	100	100	1.88

Adapted from Hulet CV, Shelton M, Gallagher JR, Price DA: *J Anim Sci* 1974;38:1201–1217.

88-1). More specifically, ewes that are bred during the summer months (e.g., June) produce fewer lambs (ovulation rate = 1) than the same ewes bred during the fall or winter months (ovulation rate = 1.7–1.8). This latter point is especially important to consider for producers who manage a program to breed ewes "out of season" (during the summer months) in an effort to produce more lambs per year.

EFFECT OF LIGHT CONTROL PROGRAMS

Reducing the exposure of ewes to long day lengths can be used to induce cyclic activity. Implementing this approach is usually achieved by blocking the exposure of ewes to the light of morning or evening through the use of "light-tight" barns. This morning/evening type of restriction attempts to minimize the exposure of ewes to the adverse effects of the high ambient temperatures associated with long days. In reality, implementing "blackout" conditions to initiate cyclic activity is seldom used due to facility requirements or limitations.

In contrast to reducing ewes' exposure to light, a radical experimental approach with considerable merit may be achieved through supplemental (artificial) lighting. This approach involves exposing ewes to artificial lights beginning on the shortest day of the year, so as to expose ewes to a total of 20 hours of light (8 hours natural plus 12 hours artificial). Subsequently, the amount of artificial light the ewes are exposed to on a daily basis is eventually reduced to 0 hours on June 21 when the natural length of day is approximately 14 hours. This paradigm effectively inverts the seasonal fluctuation in length of daylight, dictating that ewes see "long days" (i.e., more light) during the winter and "short days" (i.e., less light) during the summer, potentially resulting in the induction of cyclic activity during the summer, which is traditionally the "nonbreeding" season. The advantage that this approach offers over the light deprivation approach is the ease with which artificial light can be added relative to the investment necessary to establish "light-tight" facilities. Unfortunately, this approach is purely experimental and has received little attention; thus, its efficacy is not well established.

Certainly the advantages of a breeding scheme that relies on phototherapy (or melatonin treatments to mimic phototherapy as discussed subsequently) appear obvious and the "natural" approach, yet two inherent disadvantages exist: (1) the need to treat ewes on a daily basis and (2) the long lag time (4 to 6 weeks) from initiating treatments to initiation of cyclic activity by the ewes.

Table **88-2**

Effect of Temperature on Variables of Reproductive Importance

	HOUSING FOR RAMS AND EWES (AUGUST)	
Variable	**Outside**	**Air-Conditioned Room**
% eggs fertilized	26%	64%
% embryonic death	49%	22%
% ewes lambing	13%	50%

Dutt and Simpson, 1957.

EFFECT OF ENVIRONMENTAL TEMPERATURE

When seasons change, so does temperature. Temperature does not appear to play a major role in dictating the cyclic activity of ewes, but it does have a major detrimental effect on embryo survival, especially when temperatures are high during the week following breeding (Table 88-2). When ewes and rams were housed in a hot climate versus a cool climate, the hot weather resulted in a lower percentage of fertilized eggs, greater embryonic death, and consequently fewer ewes lambing. Any condition that limits an animal's ability to cool itself (e.g., high humidity) adversely affects reproductive performance.

EFFECT OF EWE AGE AND BREED

Breed and age also affect the breeding activity of ewes (Table 88-3). Some breeds of sheep, such as Rambouillet, Merino, and Dorset, have longer breeding seasons than others, such as Southdown and Cheviot. Fortunately, long breeding seasons are genetically dominant; therefore, it is possible to increase the length of the breeding season through selective breeding. Within a breed, as an animal's age increases, the length of the breeding season and fertility also increases (see Table 88-3). Ewe lambs that are starting to cycle for the first time generally tend to start cycling about 3 weeks after the adults and stop cycling about 3 weeks before the adults (see Fig. 88-3). As age increases, reproductive efficiency also increases when measured by number of surviving offspring (due, in part,

Table **88-3**

Effect of Age of Dam on Three Lamb Production Traits Independent of Breed, Year, Type of Birth, and Type of Mating

Age of Ewe	Number of Ewes	Fertility (% of Ewes Lambing per Ewes Exposed)	Prolificacy (% of Lambs Born per Ewes Lambing)	Overall Reproduction (% of Lambs Weaned per Ewes Exposed)
2	732	87	126	83
3	647	91	131	98
4	515	93	137	112
5	427	92	143	105
6	288	90	145	110
7	190	94	141	105
8	109	90	145	93
9+	54	82	153	99

From Sidwell et al., 1962.

to learned mothering ability) and number of offspring produced (due, in part, to an increase in ovulation rate as ewe age increases).

EFFECT OF NUTRITION

For animals to produce, they must be adequately nourished and be maintained in good body condition. Poorly nourished animals cannot meet their needs for maintenance and be expected to produce a product. Clearly, ewes with the greatest nutrient intake respond most rapidly to the onset of the breeding season and continue to respond with an increase in ovulation rate until the termination of the breeding season. In contrast, ewes receiving less than National Research Council recommendations exhibit only a modest increase in ovulation rate at the initiation of the breeding season, and fail to maintain that slight increase; most likely owing to other energetic demands.

The rapid response of ewes receiving greater than National Research Council nutritional recommendations with an increase in ovulation rate has long been known and referred to as *flushing*. In order for the technique of flushing to be effective, it is usually initiated 2 to 3 weeks prior to the beginning of the breeding season and is accomplished by supplementing ewes with a high-energy feed such as 0.5 to 1.5 lb of corn per head per day. The most effective time of the year to use the technique of flushing to increase ovulation rates of ewes is when ovulation rates are marginal and especially when ewes are transitioning into or out of the breeding season. Flushing is also most effective in ewes with greater energetic demands. As can be seen in Table 88-4, regardless of the fact that both fat and thin ewes are gaining weight at an equivalent rate in response to the flushing stimulus, only the thin ewes respond with an increase in ovulation rate. It should also be noted that the increase in ovulation rate in response to the flushing stimulus does not exceed that capability exhibited by the fat ewes regardless of energy intake. Consequently, flushing is a short-term technique that merely permits the animal to express its genetic potential to ovulate. Neither flushing nor feeding the animal in excess of its needs permits the animal to supersede its genetically defined limitations. In contrast, limiting nutrient availability not only reduces ovulation rates but also shortens the length of the breeding season, limits the animals' milking ability, and compromises other systems related to production. Simply put, when animals are not fed, they fail to produce.

Table **88-4**

Effect of Ewe Condition on Ovulation Response to Flushing

Ewe Condition	Treatment	Rate of Gain	NUMBER OF EWES OVULATING	
			Singles	*Twins*
Thin	Flushed	.23	12	8
	Unflushed	.02	20	0
Fat	Flushed	.21	10	10
	Unflushed	.04	8	12

From Clark: *Anat Rec* 1935;60:125.

EFFECT OF STRESS

The stress imposed on animals can come from disease, parasites, confinement, lactation, suckling stimulus (an effect independent of lactation), and other factors both known and unknown. Stressful situations typically reduce production performance.

When considering the stress of suckling, ewes that suckle lambs take longer to return to estrus than ewes from which lambs have been weaned at an early age (Fig. 88-4). The recipe for predicting the outcome of stress management approaches may not always be as anticipated, depending on the source of the stress. Consequently, selective removal of specific stressful stimuli can produce responses that are either ineffective, permissive, or facilitating (e.g., ram introduction, discussed subsequently) to reproductive performance. The ability to

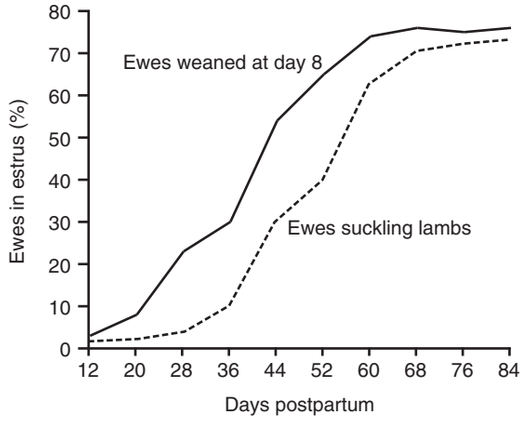

Fig. 88-4 Effect of early weaning versus suckling on ewes returning to estrus.

predict the outcome of selective stressful stimulus requires the ability to collectively assess the total impact of that stress on the balance that exists between the animal's inherent drive to reproduce and the demands that keep her from reproducing. For example, although early weaning of lambs may appear to provide a simple solution to accelerate the rebreeding of ewes, the technique is ineffective when used as the only stimulus to induce cyclic activity in ewes lambing during the spring or summer months. The differential response between groups of ewes lambing in the fall versus the summer can be attributed to the powerful inhibitory effects of long day lengths. In contrast, by creating an environment in which several inhibitory stressors are negated, using combined approaches, such as lamb removal and flushing, may be sufficient to initiate cyclic activity in ewes even in the presence of other inhibitory influences such as long day lengths.

EFFECT OF SEASON

It is likely that a far greater proportion of ewes initiate cyclic activity during the nonbreeding season in response to combined management approaches than is realized. Ewes initiating cyclic activity following a period of anestrus typically exhibit a silent first ovulation. Consequently, producers fail to realize the impact of their actions and relax their efforts to rebreed ewes, thus permitting the inhibitory influences to again suppress the ewe's drive to reproduce. Should these efforts occur during the influence of positive environmental conditions, behavioral estrus will follow silent ovulations within 3 weeks. During transitional phases (e.g., from anestrus to estrus or from prepuberty to puberty), a hastening of the onset of breeding activity can be achieved in a greater proportion of ewes with techniques such as short-term lamb removal and flushing. Some reports also cite the positive effects of ram introduction and transportation stress on inducing ewes to ovulate. Management of transportation stress to evoke a positive response is likely among the most difficult to assess because of the delicate perception of transportation as a stimulatory as opposed to an inhibitory stimulus.

EFFECT OF RAM INTRODUCTION

The most powerful stimulus that is widely known and used is the introduction of a novel ram. In general, to evoke a ram-introduction response, ewes need to be isolated from the sight, sound, and smell of rams for approximately 30 days prior to introduction, although reports in the literature vary greatly with regard to the completeness and duration of isolation. Typically, the ram-introduction technique involves the use of teaser rams to hasten the onset of the breeding season. Once ewes are cycling, fertile rams are introduced. When attempting to induce estrous cycles in anestrous or prepubertal ewes using the ram introduction effect, it is recommended that the planned herd sires and not teaser rams be used because the response of ewes may be short-lived. Fertile mating should be encouraged at every opportunity because the likelihood of nonpregnant ewes recycling following a ram-induced ovulation during anestrus is low.

HORMONE THERAPIES USED IN BREEDING SCHEMES

Hormonal manipulations of the reproductive processes offer many advantages that are difficult to attain without their use. These advantages include concentration of the breeding and lambing seasons, permitting (1) more efficient use of labor and facilities and (2) greater supervision of ewes and lambs at lambing, thus providing (a) greater opportunity for less death loss and (b) greater success in cross-fostering lambs. Concentrated breeding and lambing also permits ewes and lambs to be managed more uniformly in flock health, nutrition, management, marketing, and processing activities. The use of hormones to induce estrus and ovulation can facilitate early breeding of ewe lambs, out-of-season breeding, potential to yield a greater litter size, and the use of artificial insemination and embryo transfer.

The advantages of exercising hormonal control over the reproductive processes may be appealing but the liabilities often include additional inputs and in some cases pose potential risks. Aside from the added costs of hormonal treatments, considerable thought and planning must be given to ensure that sufficient ram power is available for synchronized breeding. Furthermore, a synchronized estrus certainly does not imply greater fertility, and at times, therapies can and do impair fertility. It should also be noted that synchronous breeding of ewes results in a relatively synchronous parturition; consequently, sufficient facilities and equipment must exist and be prepared ahead of time for this outcome. Producers should be cautioned that concentrating the lambing of ewes in a common environment will yield greater opportunity for newborn lambs to be orphaned or mismatched with their dam.

Unquestionably, effective hormonal therapies have been established to control the reproductive processes of ewes. Most therapies are available for commercial use in

the major sheep-producing countries, only a few are available in Canada, and none have been approved for commercial use in the United States. The inability to take advantage of pre-existing hormonal therapies may be frustrating to U. S. producers, but it necessitates that greater emphasis be placed on culling animals for poor reproductive performance in order to remain competitive. Hormonal control of the reproductive processes should never be used as a substitute for good management practices. In fact, in species in which hormonal therapies are available for reproductive management, one may question whether producers are actually making reproductive progress or are inadvertently propagating the evolution of animals that ultimately will require hormonal intervention to reproduce!

HORMONE PROGRAMS

The challenges of most programs for hormonal control of breeding are (1) to synchronize the estrous cycles of ewes during the fall and winter breeding seasons; (2) to induce a fertile estrus during the late winter and early spring anestrous period; and (3) to advance the breeding season into the summer transitional period.

Thus, the goals of the producer will require a management decision to be made based on the season of the year. In other words, the producer must determine *what proportion of ewes are cycling versus noncycling*. One approach to determine this is to check all ewes for estrus with teaser rams at least one cycle length (average 16.5 days) prior to administering treatments. If the entire flock is cycling, greater than 95% of the ewes will be in estrus within a 16.5-day period. This means that approximately 6% of the ewes in the flock should be in estrus each day (100% ÷ 16.5 days = 6%/day). Alternatively, rather than determining the proportion of ewes that are cycling throughout one cycle, another approach, albeit potentially less accurate, would be to determine the proportion of ewes in estrus each *day* as a guide to estimate the proportion of ewes in the flock that are cycling. Note, however, that failure to detect estrus does not imply that ewes are not cycling. Conversely, not all ewes that are marked or stand to be mounted by a ram are cycling (e.g., up to 28% of ewes that conceive at a previous estrus will subsequently show estrus). Another option, albeit less producer "friendly," is to measure plasma or serum progesterone. Elevated progesterone concentrations in a single sample will identify 70% to 80% of ewes with corpora lutea (CL). Two samples, taken 8 to 10 days apart, enable identification of virtually 100% of ewes with CL, which is indicative of the fact that the ewes are either cycling or pregnant.

SYNCHRONIZATION OF ESTRUS IN CYCLING EWES

Inducing Luteolysis

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the preferred means for synchronizing estrus and ovulation in cycling ewes (Table 88-5). Fertility of ewes following a $PGF_{2\alpha}$-synchronized estrus does not differ from that observed in ewes bred at a spontaneous estrus. The mechanism of action of $PGF_{2\alpha}$ is to induce regression of CL found in cycling and pregnant ewes; therefore, it is completely ineffective in ewes without CL, such as anestrous and prepubertal ewes. When a single $PGF_{2\alpha}$ injection is given to a flock of cycling ewes, 60% to 70% of the ewes in the flock exhibit synchronized estrus 30 to 48 hours after treatment. The actual interval from treatment to estrus depends on the day of the estrous cycle when ewes are treated. The proportion of ewes that fail to respond to $PGF_{2\alpha}$ do so because (1) ewes are not cycling, (2) ewes are in the process of forming new CL—when they are known to be unresponsive to $PGF_{2\alpha}$, (3) ewes are in the process of regressing the current CL, or (4) ewes are in the late stages of pregnancy, when they are also known to be unresponsive to $PGF_{2\alpha}$. Ewes in the third category exhibit estrus during the interval between $PGF_{2\alpha}$ treatment and the synchronized estrus. If complete synchronization of the flock is desired, such as for timed artificial insemination, this can be achieved by giving two $PGF_{2\alpha}$ treatments 9 to 11 days apart. The greatest proportion of ewes (virtually 100%) will be synchronized following the second injection.

A conservative approach to concentrate the breeding season of a flock of cycling ewes (using less $PGF_{2\alpha}$ and yielding less synchronization) requires that a $PGF_{2\alpha}$ treatment be given on the fourth day of a 7-day breeding period. This approach is as follows: during the first 4 days of this approach, ewes that exhibit estrus are bred. On the fourth day, ewes that were not bred should be treated with $PGF_{2\alpha}$. During the 3 days following $PGF_{2\alpha}$ treatment, the remainder of the ewes should exhibit estrus and be bred. Using this approach, all ewes should be bred within 7 days.

Delaying Estrus and Ovulation

Progestins are commonly used in synchronized breeding programs as an effective means of controlling estrus and

Table **88-5**

Prostaglandin Analogs Used to Synchronize Estrus During the Ovulatory Season in Sheep*

Product	Drug	Dosage	Source
Lutalyse	Dinoprost	15 mg at 9- to 11-day interval	Upjohn Co, Kalamazoo, MI
Estrumate	Cloprostenol	125–150 μg at 9- to 11-day interval	Bayer, Animal Health, Shawnee Mission, KS

*Note: Prostaglandins are not approved for use in sheep in the United States.

ovulation. Progestins are effective in cycling ewes but are less effective in noncycling ewes without additional treatment. Progestins can be used in situations in which ewes are potentially pregnant without yielding any detrimental effects on the fetus. The effectiveness of a progestin to serve as a synchronization agent can be judged by (1) its ability to delay estrus and ovulation, (2) the means by which it must be administered, (3) the length of time it must be administered, (4) how rapidly it can be removed from the ewe's system, and (5) its effect on subsequent fertility. The ability of a progestin to delay estrus and ovulation is a function of the potency of the progestin and the dose administered. Progesterone, a natural progestin, is effective in delaying estrus and ovulation, but large doses are required. As a result, synthetic progestins have been developed that are more potent than progesterone. The various properties of the progestins have permitted the use of several approaches to deliver progestins to ewes: (1) daily or twice-daily injections, (2) intravaginal sponges or pessaries, (3) implants of various sizes placed subcutaneously, and (4) feed additives. Although each delivery system has its advantages and disadvantages, the approach used must be capable of continuously maintaining the minimal effective concentration necessary to delay estrus and ovulation for the duration of treatment.

Progestin-Impregnated Vaginal Pessary

Pessaries are inserted with a clean, lubricated speculum. Cleanliness is essential to avoid vaginitis. Rough handling or lack of lubrication causes vaginal irritation and adhesion of the sponge to the mucosa. Many specula have pointed ends that can irritate or injure. Proper restraint is important to further ensure that no injury occurs. Improperly placed pessaries are often introduced into the urethra or through the vaginal wall. Producers report a fall-out rate of pessaries to be between 2% and 25%. On occasion, the string attached to the sponge becomes entrapped in the vagina and the producer may mistakenly believe that the pessary has fallen out. A ewe without the string exposed should be checked for the presence of a retained pessary. Maiden ewes often have vaginal strictures or hymenal remnants that can be damaged, resulting in mucosal injury and adhesions and often allowing the pessaries to adhere to the vaginal wall. The presence of a pessary results in accumulation of vaginal fluids in the vagina that drain upon removal. Rams will be attracted to these ewes and attempt to breed, thus potentially reducing sperm available for breeding at the ovulatory estrus.

Controlled Intravaginal Drug-Releasing Devices

Controlled intravaginal drug-releasing devices (CIDRs) are an alternative to the vaginal pessary. CIDRs contain natural progesterone impregnated into the silicone. The device is easier to insert than a pessary and fewer losses are reported. Reports on fertility comparing pessaries with CIDRs show no difference. Less vaginal irritation (with less accumulation of vaginal fluid) occurs with CIDR use.

Norgestomet Implants

Norgestomet (Syncro-Mate-B) implants are inserted subcutaneously in the back of the ear. Some will dislodge if not inserted to sufficient depth. Implants are removed by making a small incision at the base of the implant and expressing the implant. A single implant contains 6 mg norgestomet. Normally, only one half of an implant (3 mg) is used in sheep. Implants normally are left in place for 10 to 14 days.

Oral Progestagens

The only orally active progestin available for livestock is melengestrol acetate (MGA). Feeding of MGA daily (0.125 mg fed twice daily for 8 to 14 days) produces a somewhat less synchronous occurrence than that achieved by norgestomet implants, pessaries, or CIDRs.

Injectable Progesterone

Progesterone in oil has been given as an IM injection of 5 to 25 mg daily.

INDUCTION OF FERTILE ESTRUS IN ANESTROUS OR TRANSITIONAL EWES

During late winter and early spring, an interval of progestin administration followed by treatment of ewes with gonadotropins induces fertile estrus in most ewes. Any of the progestin products can be used (Table 88-6). Most

Table **88-6**

Progesterone or Progestagen Products Used to Synchronize Estrus*

Product	Drug	Location	Days	Source	Country
Veramix pessaries	60 mg medroxyprogesterone acetate	Vagina	14–16	Upjohn	Canada
Cronolone pessaries	30 and 40 mg flurogestone acetate	Vagina	14–16	Intervet, London, Ontario	Canada
CIDR-G	330 mg progesterone	Vagina	14–16	AHI Plastics	Australia, New Zealand
MGA	Melengestrol acetate	Feed: 0.125 mg bid	8–14	Upjohn	United States
Syncro-Mate-B	3 mg norgestomet (one half implant)	Ear implant	10–14	Sanofi, Inc, Overland Park, KS	United States

***Note:** Most of these products are not approved for use in sheep in the United States.

Table **88-7**

Gonadotropin Products Used in Sheep

Product	Drug	Dosage	Source
Equinex	PMSG	400–500 IU in anestrus/0 to 200 IU in breeding season	Ayerst Laboratories, Montreal, Quebec, Canada
Stimukron	PMSG	Same	PVU-Sanofi Saute Animale, Montreal, Quebec, Canada
Folligon	PMSG	Same	Intervet, London, Ontario, Canada
PG-600	PMSG + hCG	200 IU + 400 IU	Intervet, Millsboro, Delaware, USA

hCG, human chorionic gonadotropin; PMSG, pregnant mare serum gonadotropin.

common choices are the progestin pessary, norgestomet implant, or the MGA feed additive. Most programs use the progestin for 8 to 14 days. The gonadotropin most widely used is pregnant mares' serum gonadotropin (PMSG, or equine chorionic gonadotropin [eCG]) (Table 88-7). PMSG induces a prolonged period of gonadotropin stimulation, primarily as a result of its follicle-stimulating hormone (FSH)–like activity with lesser and varying amounts of luteinizing hormone (LH)–like activity. It is normally administered at the time of progestin removal or within 48 hours prior to removal. An alternative, and often more readily available product in the United States, is PG600, which consists of a combination of PMSG and human chorionic gonadotropin. FSH products, traditionally used for superovulation, which have also been used when the other gonadotropins are not commercially available.

BREEDING EWES DURING THE SUMMER TRANSITIONAL PERIOD

Early in the spring transitional period, the most successful method to induce fertile estrus is treatment with a combination of progestin and gonadotropins. As ewes approach the first ovulation of the season, the ram effect can also be used successfully, both to induce ovulation and to synchronize estrous cycles. Ram exposure results in increased LH production in receptive ewes, followed by ovulation without estrus (silent ovulation) within a few days. The silent ovulation is followed by development of a CL, which either fails prematurely (about 4- to 6-day lifespan) or persists for the normal 14 days and is followed by a normal ovulatory estrus. Those ewes with premature luteal failure may experience a second silent ovulation, followed by development of a CL that persists for a normal lifespan and a normal estrus 17 days later. This results in two peaks of estrous activity at about 18 and 24 days after introduction of the teaser animal. The success of inducing estrous cycles and the proportion of ewes that follow either path depend on the breed and the time interval to or from the natural breeding season. The shorter that interval, the better the probability of a positive response.

Administration of progesterone prior to introduction of teaser rams may improve the synchrony of estrus. Vaginal pessaries, melengestrol acetate fed for 8 to 14 days, or an injection of 20 mg of progesterone in oil given the day of ram introduction has been shown to work well in conjunction with teaser rams during the transitional season. Sixteen days following ram introduction, $PGF_{2\alpha}$ can be given to yield an effective means of inducing a synchronizing estrus and ovulation in anestrous, transitional ewes.

It is generally recommended that the females be abruptly introduced to the male following isolation from the sight, sound, and odor of rams for the 30 to 60 days prior to introduction. Recently, however, it has been shown that introduction of a novel male (new to the females) works as well and may be easier than housing all males at a distance. The novel ram need only be introduced for 48 hours to have full effect. For optimum response, ewes must be weaned prior to ram introduction and be in good breeding condition.

Males can be rendered sterile by vasectomy or epididymectomy. Castrated males or cull females can be androgenized by administering 100 mg testosterone in oil weekly for 3 weeks.

MANAGEMENT OF OVULATION AND LAMBING RATES

Although some improvement in the number of lambs born can be realized by increasing ovulation rates, the relationship between ovulation rate and pounds of lamb marketed is constrained by a multitude of other factors. Although ewes have the capacity to ovulate a large number of eggs in response to hormonal stimulation, limitations exist that do not permit the survival of superfluous embryos or neonates. It should also be noted that as ovulation rates increase, the number of live offspring begins to decline (Fig. 88-5).

Management Considerations That Influence Ovulation Rates

Breed

A number of breeds, such as Booroola Merinos, Finnsheep, and Romanov, can be used in crossbreeding programs to increase ewe productivity. This approach is very powerful, is repeatable, and is the easiest to implement, yet is often the most difficult to encourage producers to adopt.

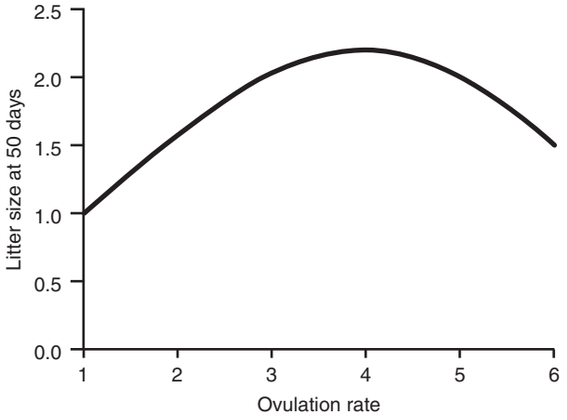

Fig. 88-5 Relationship between ovulation rate and litter size at 50 days after lambing.

Age
Ovulation rates are lower in ewe lambs than in mature ewes.

Season
Ewes bred out of season typically have lower ovulation rates than ewes bred in season.

Nutritional Status
The nutritional status of the animal directly dictates the ability or inability to express genetic potential. Flushing can be used as a short-term means of relieving a nutritional deficit and to permit ewes to express their genetically predetermined capacity to ovulate.

Active Immunization of Ewes to Increase Ovulation Rates
Immunization of ewes against reproductive hormones has been used as an approach to increase ovulation rates. Two avenues of investigation have been pursued.

Antiandrostenedione. Products for immunizing ewes against the gonadal steroid androstenedione became commercially available in some countries in 1983. These products are prepared by linking the hormone androstenedione to human serum albumin. When this complex is injected into ewes, the ewes produce antibodies against the complex, which inactivates endogenous androstenedione. Although the precise mechanism by which this approach functions to increase ovulation rate is incompletely understood, it is likely that a greater secretion of gonadotropins from the pituitary is permitted or follicular receptivity to the gonadotropins is enhanced. Following initial immunization, only a single annual booster immunization is required 3 to 4 weeks before rams are introduced. An increase in lambing rate of 0% to 47% has been observed. The response of the ewe does not appear to be dependent on the age of the ewe, but may be influenced by breed and body condition of ewes. Ewes in poor body condition show little or no improvement in lambing rates. There have been no detrimental effects of the vaccine in terms of congenital abnormalities, lamb survival, lamb growth, wool growth, or wool characteristics.

Anti-inhibin. Secretion of FSH is regulated in part by follicular secretion of inhibin, which suppresses pituitary secretion of FSH. Ewes actively immunized against inhibin exhibit increased peripheral concentrations of FSH and consequently increased ovulation rates. Although active immunization of ewes against inhibin has yet to reach commercial application, the efficacy of this approach has been well documented.

Gonadotropin Therapy
Hormones with gonadotropic properties such as LH, FSH, PMSG, and human or equine chorionic gonadotropin (eCG or hCG) function to stimulate follicular development or induce follicles to ovulate, or both. Traditionally, FSH and PMSG have been used to recruit follicular development in animals in which a superovulatory response is desired. Dosages of PMSG in excess of 600 IU have been reported to increase ovulation and lambing rates in well-managed ewes. Dosages of 750 to 1500 IU are used to cause superovulation in ewes for embryo transfer and are not recommended for ewes expected to carry pregnancies to term.

Melatonin Therapy
Melatonin has been used to alter the traditional breeding season and has been reported to be associated with increased ovulation and lambing rates.

Factors Affecting Ovulation and Lambing Rates

Many factors affect the success of a breeding program. This following list is intended as a guide for consideration in the success or failure in a breeding program.

Ewe Factors

Breed
Age
Parity
Fertility
Lactational status
Time from previous lambing
Body condition

Ram Factors

Breed
Age
Libido
Body condition
Fertility

Breeding Management

Season of year
Ram : ewe ratio
Progestin treatment: duration, handling, product
Gonadotropin treatment: product, dose, handling
Time of ram introduction
Use of teaser males
Stress at breeding
Environmental temperature before and after breeding

Diet

Ration
Flushing
Phytoestrogens in pasture, hay, and moldy feeds

OTHER HORMONAL THERAPIES USED FOR MANAGED BREEDING

Melatonin

Sheep are short-day breeders; therefore, as day length increases, breeding activity decreases. In response to darkness, the pineal gland secretes the hormone melatonin. When melatonin is administered to ewes for several weeks, it simulates the endocrine events characteristic of short days and induces ewes to initiate cyclic activity. Ewes may be given melatonin as an oral drench, as a feed additive, as an injection, or as an implant placed in the rumen, vagina, or subcutaneously. When fed, injected, or drenched, doses of 2 to 3 mg melatonin per head per day have been used and are administered approximately 6 to 8 hours before darkness. It should be realized, however, that following the initiation of melatonin treatments, the onset of cyclic activity is not immediate and may require 4 to 6 weeks before an effect is realized. Once ewes have begun cyclic activity in response to melatonin, the treatment must be continued to maintain breeding activity. Treatment of ewes with implants containing melatonin may be a more practical means of controlling breeding activity. Implants of melatonin are available in some countries. An added advantage of melatonin implants is purported to be a slight increase in ovulation rate, resulting in an increase in lamb production.

ACCELERATED BREEDING PROGRAMS

The single most important factor contributing to pounds of lamb marketed per ewe per year is the number of lambs born. With a gestation length of approximately 146 days, it is possible for ewes to produce more than one lamb crop per year. However, when considering implementation of an accelerated lambing system, it is critical to remember that if one cannot or does not do a good job of managing ewes to lamb once a year, one will not do a good job of managing ewes to lamb more than once a year. In North America, the two accelerated systems of production that are most common regiment ewes to a schedule permitting them to produce three lamb crops in 2 years (three-in-two) or five lamb crops in 3 years (the Star system; Fig. 88-6). Lambing-to-breeding intervals in these two systems are 8 months and 7.3 months, respectively. Other systems also exist, such as the CAMAL (*C*ornell *a*lternate *m*onth *a*ccelerated *l*ambing) system, which permits a lambing-to-breeding interval of 6, 8, 10, or 12 months and is accomplished by exposing ewes to rams every other month. By far, the most popular and well-studied system is the Star system.

With the Star system, the calendar year is depicted in a circular fashion and segmented into 5 intervals of 73 days each, which is also one half the gestation length of most ewes (146 ÷ 2 = 73 days). Each of the points of the star coincides with the start of that time period when a given group of ewes is exposed to rams. Each of the limbs emanating from the point coincide with when that group of ewes is scheduled to lamb (first limb) and is subsequently re-exposed to rams (the second limb). For example, if a ewe is bred on the first day of a 30-day breeding period, she should lamb on the first day of the lambing season two periods later. Concomitantly, while one group of ewes is being bred, another group of ewes is lambing. Contingent upon when they lamb, ewes lactate for 36 to 63 days before being weaned (over a 7-day interval) and subsequently are re-exposed to rams for 30 days. Ewes that fail to conceive during the 30-day breeding period are re-exposed at a subsequent breeding period, which occurs every 73 days until ewes conceive or are culled. In an ideal scenario, ewes will conceive or lamb at every third point of the star.

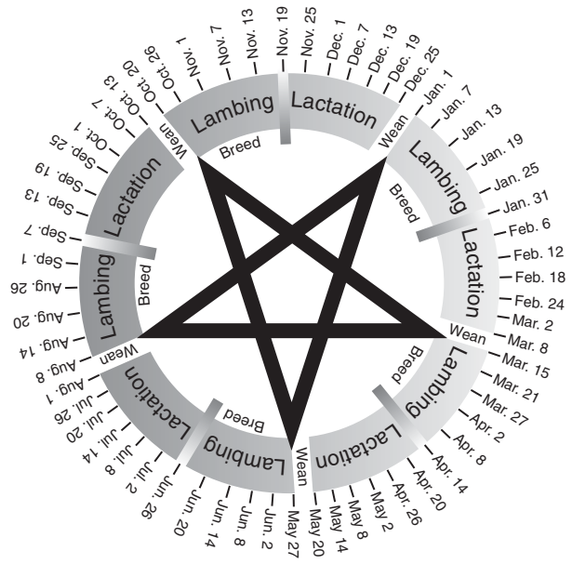

Fig. 88-6 The Star accelerated lambing system, with five annual, concurrent breeding and lambing intervals.

The advantages cited for the Star system, as with any accelerated system of production, include the following:

1. Greater reproductive performance by the ewes, permitting ewes to lamb up to five times in 3 years. In addition, this system also facilitates the identification of those ewes that will breed out of season, as well as those with a short postpartum anestrous interval.
2. Fewer rams are needed because only one fifth to one third of all ewes will be bred at any time.
3. More effective or less seasonal use of labor and facilities. Although the lambing barn need not be as large as that required to accommodate the entire flock, it must permit *efficient* feeding and waste removal. Good management skills are of the utmost importance.
4. Improved cash flow as the result of a more steady supply of marketable lambs.

Factors to consider and possibly concessions to make before implementing the accelerated system are noted in the following sections.

Genetics of the Ewe

The most important consideration or possible concession to make is in choosing the breed of sheep. The genetics of the animal has the greatest impact on the success of an accelerated system of production. Highly seasonal breeds cannot be used to constitute the genetics of the ewe flock. Maternal breeds with the least seasonal breeding influences, shortest gestation lengths, and highest ovulation rates afford the greatest opportunity for a successful accelerated system of production.

Genetics of the Ram

Considerable attention must be given to the ram or system of breeding to ensure year round performance. Typically, ram breeds are used as terminal sires in the accelerated system. Although semen production is a continuous process throughout the year, the capacity or interest of rams to mate during the nontraditional breeding season could influence the success of an accelerated program. Artificial insemination of ewes is a tempting solution but presents significant challenges.

Management of the Ewes

Early weaning of lambs (36 to 63 days post partum) may predispose ewes to mastitis, which contributes to a greater turnover rate of ewes in an accelerated system of production.

Performance of the Offspring

The offspring generated from an accelerated system of production are offered solid feed at an early age and may lack the genetic constitution to compete at the marketplace with lambs selected for carcass traits.

Pregnancy Status of Ewes

It is essential to know the pregnancy status of ewes in accelerated systems. An accurate system for early pregnancy diagnosis is essential to identify open ewes for the next breeding group.

Feed Requirements

Feed usage is typically greater in an accelerated system because more demands are placed on the ewes, and because of the added requirements generated by early weaning of lambs.

Seasonal Influences

An issue that must be reconciled is the seasonal influence on ovulation rate and neonatal or embryonic mortality rate. Typically, ewes bred out of season produce fewer lambs than those bred in season. This is of significant concern, because two of the three breedings in the 2-year system and three of the five breedings in the 3-year system occur out of season. Consequently, the issue to be addressed is whether the opportunity to produce more lambs justifies the more intense management inputs. The answer is unique to each operation. Marketing incentives must exist to foster the adoption of accelerated systems of production.

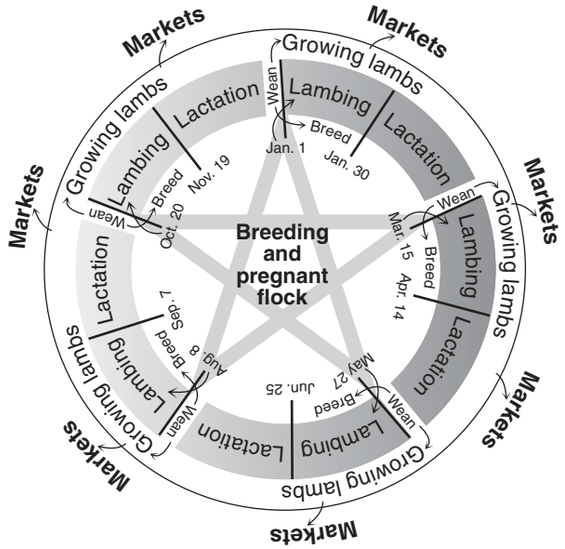

Fig. 88-7 An example of the time management decisions that must be made in an accelerated system of production.

Time Management

Developing a schedule and adhering to it is critical to the success of an accelerated system of production. At any point in time, the manager will have to simultaneously manage feeding and care of replacement ewes and rams, the ram battery, lambs in various stages of growth and development, and ewes that are being bred, lambing, lactating, or in various stages of gestation (Fig. 88-7).

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CHAPTER 89

Pregnancy Diagnosis

FULLER BAZER, WAYNE CUNNINGHAM, and DEBORAH MARSH

Pregnancy diagnosis in sheep is an important management practice because of the impact of reproductive performance on economic return from the flock. Early pregnancy diagnosis, determination of fetal numbers and estimation of day of gestation, and recognition of abnormalities of pregnancy can provide producers with significant management opportunities to enhance reproductive efficiency. Historically, producers of small ruminants have had few options with regard to pregnancy diagnosis. They have been forced to wait a sufficient period of time to visibly detect return to estrus or to detect pregnancy based on mammary development and abdominal enlargement. The latter method, although relatively accurate in multiparous females, can give false negative results in primiparous females and can only be performed with a high degree of accuracy during the latter stages of gestation. Earlier diagnosis based on return to estrus following mating has commonly relied on use of a vasectomized ram with brisket paint or fitted with a crayon marking harness. The nonpregnant ewes that continue to cycle during the breeding season are marked by the teaser ram. However, some pregnant ewes will continue to exhibit estrus and be marked by the teaser ram. High-libido teaser rams may mark pregnant ewes that are not exhibiting estrus, as well as nonpregnant ewes not exhibiting estrus.

TRADITIONAL OR HISTORICAL METHODS OF PREGNANCY DIAGNOSIS IN EWES

Radiography

This technique has been used effectively for rapid testing of up to 400 to 600 ewes per day for pregnancy diagno-

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TRADITIONAL OR HISTORICAL METHODS OF PREGNANCY DIAGNOSIS IN EWES

Radiography

This technique has been used effectively for rapid testing of up to 400 to 600 ewes per day for pregnancy diagno-

sis and determination of number of fetuses; however, the cost of the equipment and risk to operator and animal health makes radiography impractical.

Rectal Abdominal Palpation

This technique requires a lubricated glass rod (1.5 by 50 cm) to be inserted into the rectum of ewes lying on their back. With the operator's hand on the abdomen and movement of the rod with the other hand, the attempt to diagnose pregnancy was done without a high level of accuracy until late in gestation. Although cheap, simple, and quick, this technique has too low a rate of accuracy to be useful.

Concentration of Progesterone in Blood

Determination of the concentration of progesterone in blood using an enzyme immunoassay provides a reliable method for detecting pregnancy as concentrations are expected to be high for pregnant ewes that maintain functional corpora lutea. However, this method is not reliable for diagnosing the absence of pregnancy due to embryonic death, uterine pathology, or failure of the corpus luteum to regress at the expected time. This method can allow one to estimate the presence of multiple fetuses as the increase in number of corpora lutea is associated with higher concentrations of progesterone in blood, but one cannot accurately estimate day of gestation or the exact number of fetuses.

Concentrations of Estrone Sulfate in Blood

This method has not proved to be useful because it is most accurate from after day 70 of gestation and does not provide useful information with respect to number of fetuses or estimates of day of gestation.

Pregnancy-Specific Protein B in Blood

Pregnancy-specific protein B (PSPB) from binucleate trophectoderm cells of conceptuses of ruminants is detectable in blood by about days 18 to 19 after onset of estrus. PSPB assays have been reported to provide 100% accuracy in detecting pregnancy, but only 83% accuracy in detecting nonpregnancy between days 26 and 106 of gestation.

Interferon Tau

Interferon tau is the pregnancy recognition signal in ruminants that acts to inhibit expression of estrogen and oxytocin receptors by uterine epithelia to prevent their release of luteolytic pulses of prostaglandin F_2 alpha ($PGF_{2\alpha}$). Many unsuccessful attempts have been made to detect interferon tau in circulating blood and lymphatic vessels. However, there are reports of increases in expression of some interferon-stimulated genes in immune cells in blood of sheep (Mx protein) and cattle (2′,5′-oligoadenylate synthase). The use of these interferon-stimulated gene products has not been proved reliable as an indication of pregnancy because any type I interferon, for example, as a result of infection or other pathology, may cause increases in expression of these interferon-stimulated genes. With respect to pregnancy diagnosis, the risk for false positives is expected to be high.

ULTRASONOGRAPHY

The inability of traditional and historical diagnostic methods to provide early, accurate pregnancy information has led to the development of alternate techniques. During the past 20 years, various types of ultrasonographic systems have been developed to diagnose pregnancy in small ruminants. Ultrasound can be defined as high-frequency sound waves above the audible range. The frequency of these sound waves is described in cycles per second, or hertz (Hz). Frequencies in the range of 20 to 20,000 Hz can be detected by the human ear; however, frequencies used for diagnostic ultrasonography are typically in the range of 2 to 10 million cycles per second, or megahertz (MHz). Two basic types of ultrasonography have been used for pregnancy diagnosis, amplitude depth (A-mode) and brightness (B-mode).

A-Mode Ultrasonography

A-mode systems are based on two different principles: sonar and Doppler shift. A-mode systems that utilize the sonar principle rely on echoes to detect differences in impedance between tissue interfaces that are separated by fluid. Accuracy is in the 80% to 85% range from day 60 of gestation to term. A-mode systems that utilize the Doppler shift principle produce a signal (either light or audible sound) when sound waves rebound from a vibrating surface, such as the maternal middle uterine artery or the fetal heart. The frequency of the waves reflected from the vibrating surface is different from that transmitted. Although Doppler systems should identify multiple fetuses by detecting two or more fetal heartbeats, actual results in practice have been poor. Depending on operator experience, stage of gestation, and animal preparation, the accuracy of the Doppler systems ranges from 70% to 90%.

B-Mode (Real-Time) Ultrasonography

In recent years, B-mode or real-time ultrasonography has become the preferred means of pregnancy diagnosis for small ruminants. Real-time ultrasonography gets its name from the appearance of image movement. The impression of movement is given by the rapid change of the image as it is displayed and is analogous to an animated cartoon. In addition to determining pregnancy status, the image of the gravid uterus produced by real-time ultrasonography allows the operator to count fetuses, determine stage of gestation, and evaluate the health status of the pregnancy. Competent operators can provide this information rapidly (250 to 300 ewes per hour) with 95% to 98% accuracy.

Real-time ultrasonographic systems include a handheld transducer and an electronic control unit with a cathode ray tube (CRT) monitor. An electrical current passes through one or more piezoelectric crystals con-

tained in the transducer and is converted into short (1 μsec) bursts of sound. Between bursts, the transducer acts as a receiver and converts the returning ultrasound waves into electrical impulses that produce a two-dimensional image on the CRT monitor. The brightness of the image depends on strength of the returning signal, which is determined by both the position and the density of the reflecting tissues.

Intrarectal and transabdominal placements of the probe for ultrasonographic scanning are used for pregnancy diagnosis in small ruminants. For transabdominal scans, the transducer is placed in contact with the area of the abdomen in the inguinal region next to the udder that lacks wool. Ultrasound waves will not pass through air; therefore, a coupling agent (such as carboxymethylcellulose) is necessary to ensure proper contact between the transducer and tissue. Wool, dirt, grease, and feces disperse sound waves, resulting in an inferior or nonreadable image.

Pregnancy diagnosis in small ruminants typically utilizes ultrasound frequencies from 3.5 to 7.5 MHz. The lower frequencies provide deeper penetration into underlying tissues but poorer resolution, and higher frequencies do not penetrate as deeply, but provide greater definition of superficial structures. A 5-MHz transducer is most versatile and provides a good diagnostic image for either transabdominal or intrarectal scanning. A 3.5-MHz transducer works well for transabdominal scans, but a 7.5-MHz transducer is preferred for transrectal diagnosis of early pregnancy and examination of ovarian structures.

There are two basic types of B-mode ultrasonographic transducers: linear array and sector.

Linear Array Ultrasonography

Linear array transducers tend to be more versatile for veterinary practitioners who use ultrasonography in a variety of species and for multiple applications. For pregnancy diagnosis, the physical size and shape of the linear transducer lends itself to both intrarectal scans of the abdomen and transabdominal scans. The linear array transducer contains a number of piezoelectric crystals arranged in a line to emit sound ("fire") sequentially and then receive the rebounded sound waves to produce a rectangular image.

Intrarectal scans provide excellent resolution and definition of fetal and placental structures and can be used to diagnose pregnancy from as early as day 18 to as late as day 120 of pregnancy. However, it should be cautioned that very early pregnancy diagnosis and fetal counts prior to day 45 of gestation may not correlate well with lambing rates, as ewes are at greater risk of fetal loss during the early stages of gestation.

The accuracy of counting fetuses using rectal scans is limited because the entire uterus cannot be examined and the operator may experience difficulty in maintaining orientation for accurate counting of fetuses. As pregnancy advances beyond day 60, accuracy in counting fetuses increases, but as the gravid uterus moves further ventral and cranial with advancing gestation, it becomes more difficult to advance the probe sufficiently to scan the entire uterus.

With the ewe restrained in dorsal recumbency, rectal scanning using a 7.5-MHz transducer is an excellent means for examining ovarian structures (corpora lutea and follicles), and for diagnosis of very early pregnancy. In this position, the reproductive tract lies against the transducer, allowing one to obtain a complete image of the uterus and ovaries.

Linear array transducers can also be used effectively transabdominally to diagnose pregnancy between days 35 and 100 or more of gestation. Operators can determine pregnancy status and fetal number, but accurate fetal counts require patience and experience. The entire uterus usually cannot be imaged with a single placement of the transducer, so the operator must remember fetal orientation to ensure that the same fetus is not counted twice.

Sector Ultrasonography

In general, sector transducers are less versatile than linear transducers, but are preferred for transabdominal pregnancy diagnosis. Small sector transducers can be used intrarectally in large species for reproductive diagnosis, but are unsuitable in small ruminants. Sector transducers are available with different scan angles and can facilitate greater scanning speed and greater fetal count accuracy. Ninety-degree angle transducers provide excellent quality images, similar to those produced by linear transducers, but require more effort and time for accuracy. Wide-angle transducers provide a complete image of the gravid uterus and permit rapid fetal counts and estimation of stage of gestation. Although resolution is reduced with the wide-angle transducer, the image is of sufficient quality to achieve accurate pregnancy diagnosis and determination of fetal numbers. Between 45 and 90 days after breeding, experienced operators can easily scan 250 to 300 ewes per hour with greater than 95% accuracy.

Pregnancy detection using a sector transducer is limited to days 30 to 120 of gestation. Early pregnancy diagnosis requires scanning high in the inguinal region, as the uterus has not changed from its nongravid position, particularly in primiparous ewes. As pregnancy progresses beyond 90 days, fetuses grow rapidly and it becomes more difficult to view the entire uterus clearly. In addition, fetal bone structures become very dense by days 110 to 120 of gestation and they scatter reflected ultrasound waves to prevent them from forming a readable image. Thus, after day 110 of gestation, fetal counting is difficult and less accurate than during earlier stages of gestation.

Sector-Linear Ultrasonography

Recent advances in ultrasonographic equipment have combined the features of linear and sector transducers. Some of the newer systems can also store images for later recall and comparison or for transfer to remote computers for analysis and image enhancement. Regardless of the type of equipment used for pregnancy diagnosis, it is important to identify the time during gestation when the examination will provide the greatest amount of useful and accurate information. That optimal period may vary according to the goals of the producer and intended application of the ultrasonographic information provided. As a general rule, the ideal time for ultrasono-

graphic pregnancy diagnosis is between days 45 and 90 of gestation. Therefore, it is important to know when the ram was with the ewe flock. Eighty days after introduction of the ram is optimal for accurate diagnosis and speed (a two estrous cycle breeding season plus 45 days).

Interpretation (Sector Scanner)

Ultrasonographic images are created by the echogenicity of the structures being scanned. Depending on tissue density, the image will include all shades of the gray scale and range from black (anechoic or nonechogenic) to white (highly echogenic). Fluid (urine, allantoic fluid, and amniotic fluid) is anechoic and appears black on the CRT. Developing bone is highly echogenic, reflecting most of the ultrasound waves, and thus appears white.

Pregnancy is diagnosed based on three primary images: fluid, placentomes, and fetal structures. Fluid in the trophoblastic vesicle is the earliest indication of pregnancy. This fluid can be visualized using a sector scanner on about day 30. The fluid-filled vesicle continues to be the most prominent sign of pregnancy until day 45. The fetus can be identified, and, with careful observation, the fetal heartbeat can be visualized at day 35. Cotyledons begin to develop around day 22 and by day 40 appear as small gray C- or O-shaped structures around the edges of the fluid-filled vesicle. By day 45, skeletal structures are sufficiently dense to reflect most of the sound waves, creating a very bright image. As gestation progresses, the fetus continues to develop and specific structures become more easily identifiable.

It is important to remember that the image produced is two-dimensional. The sound path is roughly analogous to a sheet of paper (a plane) passing through, or "slicing," a structure. Thus, the difference in the appearance of the structure examined is related to the angle at which the sound beam penetrates the structure. For example, rotating the transducer 90 degrees will alter a longitudinal view of a fetal trunk to a cross-sectional view.

Determination of crown-rump length or biparietal diameters with electronic calipers and charts gives an accurate (±1 to 2 days) indication of fetal age. Changes in fetal size and development tend to remain relatively consistent through day 80 of gestation, regardless of the number of fetuses. Therefore, if nutritional requirements of the pregnant ewe are being met, fetal structure measurements provide an accurate indication of day of gestation. This technique is, however, time-consuming and is best used to teach operators to accurately estimate day of gestation. A more rapid estimation of day of gestation compares the size of a cross section of the fetal trunk to the size of a series of circles predrawn or precut to correlate with specific days of gestation. This guide is placed next to the CRT for quick comparison with the ultrasonographic image.

Counting fetal numbers accurately depends on the stage of gestation, the transducer used, and the technician's expertise. Image interpretation for multiple fetuses depends on visualizing at least two fetuses with a septum separating them. The cotyledons (C-shaped) open into the uterine horn toward the fetus they supply. The septum between fetuses is often seen with cotyledons opening in opposite directions. Because the uterus of a ewe with multiple fetuses develops more placentomes than a ewe with single fetus, the operator can surmise that there is more than one fetus present by simply noting increased numbers and concentration of placentomes. After day 90 of gestation, the fetus and uterus enlarge to such an extent that it is difficult to scan the entire fetus or fetuses and make accurate counts. Counting triplets and quadruplets is generally less accurate and more time consuming.

Once an operator understands the images that allow accurate diagnosis of pregnancy, the images for accurate diagnosis of the nonpregnant state must be learned. A nonpregnant ewe tends to cause the operator to be apprehensive in the absence of classical signs of pregnancy. Transrectal linear array transducers provide an image of the nongravid uterus, but a sector transducer does not generally produce an interpretable image of the nongravid uterus. However, when an operator using a sector transducer completes a thorough, methodical scan that results in a bland gray image, the diagnosis of nonpregnant is justified. It is the wise operator who classifies a ewe as nonpregnant before day 30 of pregnancy. Establishment of a methodical scanning technique will assure that the operator considers all possibilities and conditions. When scanning uteri of ewe lambs and ewes in early pregnancy, the operator can achieve the best examination with the transducer high into the inguinal area. In contrast, during late pregnancy, the best results are obtained by placing the transducer as far cranial and toward the midline as possible while still maintaining adequate contact with skin that is free of wool. Regardless of stage of pregnancy, scanning the uterus both transversely and longitudinally improves accuracy, particularly when counting fetal numbers. With experience and scanning during optimal stages of gestation, diagnostic images can be obtained within a few seconds.

Errors and Artifacts

Operator error can easily occur at either end of gestation. Early pregnancies can be missed if the transducer is not placed high enough in the flank. Late stage pregnancies can be overlooked if the scan is confined to the inguinal area and does not extend cranially and ventrally on the abdomen. Artifacts may result from improper contact due to material interposed between the transducer and skin. Structures such as the bladder may also confuse the inexperienced operator as it is seen as a circumscribed fluid-filled structure just cranial to the pelvis. Rumen fill can displace the uterus from its anticipated location and result in the operator mistakenly identifying a pregnant ewe as nonpregnant. Rumen gases also tend to produce a distorted image.

There are several explanations for lambing results not correlating with the ultrasonographic diagnosis. Operator error is assumed to be the most frequent source of error. In some situations this is justified, but in other situations the operator may be unjustly accused. One situation mentioned previously is that of embryonic/fetal death before day 40 of gestation. Another situation is loss of a twin owing to nutritional deficiencies, disease, or some unidentified cause. Advising a client of these possibilities is an important part of establishing credibility as a com-

petent operator. Providing the producer with the opportunity to observe ultrasonographic images on the screen and offering an explanation of the images can be an invaluable client teaching tool.

Ultrasonographic Pregnancy Diagnosis Justification

Real-time ultrasonography provides four types of information: (1) early diagnosis of pregnancy, (2) number of fetuses, (3) estimated day of gestation, and (4) abnormalities associated with the pregnancy. However, it is important that both veterinarians and their clients remember that the real value of this technology is realized only when the information provided is used to increase the efficiency (reproductive and economic) of the sheep operation. Defining and understanding the producer's goals will indicate whether pregnancy diagnosis will be beneficial.

Early diagnosis of pregnancy. Early pregnancy determination provides producers with opportunities to make important economic decisions. Identification and early culling minimizes the investment in nonproductive ewes and provides the opportunity to replace them with productive ewes. Results from one study determined that the cost of maintaining one nonpregnant ewe through the entire production year is equivalent to the profits from six to seven productive ewes.

Ewes not mated to the desired ram or not intended to be bred can be identified early in the breeding season and corrective actions taken to either terminate pregnancy and rebreed to the desired ram or, in the case of ewe lambs, manage them to enhance lambing rates.

Ewe lamb breeding programs can be managed more effectively. Ewe lambs that exhibit early sexual maturity and become pregnant can be identified with ultrasonography. Increasing this characteristic in a flock increases lifetime profitability of ewes. Ewe lambs that do not become pregnant can be marketed before 1 year of age.

Pregnant ewe lambs entering feedlots can be identified early and separated from their contemporaries and either marketed prior to lambing or observed more closely.

Fetal numbers. The most important economic parameter for sheep operations is pounds of lamb sold per ewe in the breeding flock. Optimizing nutritional status of ewes with multiple fetuses will help ensure that more lambs are born and survive to weaning and market. Providing an increased quantity and quality of forage to multiparous ewes will enhance implantation rates for conceptuses, placental development, fetal growth and development, birth weights, and survival of neonates. Proper nutrition also helps prevent pregnancy toxemia, increase milk production, and enhance mothering ability of ewes.

Stage of gestation. Estimating the day of gestation helps producers plan their lambing season needs based on the entire flock and individual ewes. Grouping ewes at similar days of gestation stage reduces labor and facility requirements during lambing, as well as allowing for more efficient nutritional management.

Identification of abnormal pregnancy conditions. Ultrasonographic examination for pregnancy can be an important diagnostic tool. Impending abortions can be identified prior to expulsion of aborted fetuses. In the case of campylobacteriosis, intervention with antibiotics can potentially prevent extensive abortion losses. Border disease virus and bovine viral diarrhea infections cause characteristic changes that can be identified with ultrasonography, as do effects of some poisonous plants (e.g., astragalus and water hemlock). When ultrasonographic findings suggest greater numbers of lambs than are actually born, fetal losses resulting from nutritional deficiencies can be suspected.

SHEEP HANDLING AND PREPARATION

Once an operator becomes proficient in interpreting ultrasonographic images of pregnancy, the foremost constraint to the number of ewes that can be scanned per hour is the handling of sheep and the movement of sheep through an efficiently designed scanning chute.

Minimizing Stress

When sheep are excessively stressed, they do not tend to move quickly and easily through the scanning area and are less than cooperative during the scanning procedure. The key elements to minimizing stress are (1) properly designed facilities that facilitate each movement of ewes; (2) handlers that understand and use good basic sheep psychology; (3) the absence of dogs to avoid their tendency to bark and bite at the sheep, causing ewes to become anxious and attempt to escape when the transducer is applied to their inguinal area; and (4) withholding feed and water from ewes for 12 hours prior to scanning to enhance handling and make scanning much easier for the operator.

Restraint

A variety of devices can be used to provide adequate restraint for scanning. The choice of the most appropriate cradle or chute depends on the type of machine and transducer being used. Rectal transducers require that the caudal aspect of the ewe be readily available, while transabdominal scanning requires easy access to the inguinal region of the ewe. Designing light, portable handling equipment can significantly reduce stress on both human and animal. Occasionally, it is easier to have the client manually hold ewes when there are only a few sheep to be scanned, but caution should be taken to ensure that equipment is protected from possible damage when performing scans on handheld sheep.

Scanning ewes with a sector scanner can be accomplished in several ways, depending on the operator's preference and the movement of sheep. Using a ramp to elevate ewes several feet above ground level in a restraining device allows the seated operator easy access to the inguinal area of the ewe. Some operators prefer to sit facing backward on the right side of the ewe, and others prefer to face forward on the left side of the chute and reach between the rear legs of the ewe. When large numbers of ewes are to be scanned, operator comfort is paramount. Protection from inclement weather might include a tent with a heater. A small tent also has the advantage of obscuring bright sunlight, which interferes with the operator's ability to see the image on the CRT.

Proper design of the working facilities helps keep sheep flowing to the scanning cradle. Owen Wright of Burley, Idaho, designed a system that allows ewes to move as fast as the scanning operator is able to accurately read the images. His design consists of two solid panels 12 ft long leading to the scanning cradle. A small ramp located at the back of these two panels allows ewes to easily enter the space just behind the scanning cradle and then step up about 6 inches onto the cradle floor. The ewe is not restrained except for a closed head gate to prevent forward movement of the ewe, but the ewe is able to see through the head gate. In front of the scanning chute are two more solid panels, which accommodate two ewes. The lead ewe is marked according to her pregnancy status and the second ewe draws the attention of the ewe being examined. Identifying ewes and separating them into groups at the time of examination reduces handling and increases assurance that ewes are properly grouped according to the ultrasonographic findings. When the ewe being scanned is released, she naturally wants to follow the ewe immediately in front of her, and the ewe waiting to enter the scanning chute quickly follows the departing ewe. With this system, 300 to 400 ewes can be scanned per hour. Wool cards placed on the inside of the chute opposite the operator helps anchor ewes to the chute. Further, the use of a plywood spacer reduces available space for movement of ewe lambs and smaller ewes to keep the ewe close to the operator. A breastplate centered on the floor toward the front of the chute helps keep ewes from kneeling while the operator is performing the scan.

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CHAPTER 90

Abortion in Sheep: Diagnosis and Control

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CLINICAL PRESENTATION

Pregnancy loss in the ewe before day 12 of gestation results in an early return to estrus when it occurs during the breeding season. Later pregnancy loss will manifest variably as one or more of the following:

- Return to estrus
- Failure to lamb in the absence of clinical signs of abortion
- Presence of a blood-tinged vaginal discharge with no fetus or placenta found
- Occurrence of abortion with identification of a fetus and/or placenta
- Birth of a stillborn and/or weak lamb at term (>142 days of gestation)

INVESTIGATION OF ABORTION

In most flocks, the proportion of sheep visibly aborting generally is less than 2%. The accepted proportion of sheep visibly aborting in a flock should be less than 5%. The abortion rate can be calculated as follows:

Abortion rate
= (number of ewes aborting)
÷ (number of ewes pregnant or exposed to the ram)

Rates exceeding 5% or a clustering of abortions in time or location (e.g., pen or farm) suggests the need for a more thorough study. Chronic abortion rates between 2% and 5% suggest an endemic problem that may require investigation. Diagnostic accuracy rates of 0.30 to 0.40 are possible when a careful investigation is carried out.

History

The following factors may be important in determining the cause of abortion:

Number and percentage of animals aborting
Animal movements—new additions or sharing of rams with another flock
Previous abortions and diagnoses
Age of sheep aborting (i.e., ewe lambs versus older ewes versus whole flock)
Specific pen or group or breed aborting
Stage of gestation at abortion (e.g., early pregnancy loss versus only late-gestation abortions)
Vaccination and nutritional history (e.g., salt or mineral supplementation or toxic weed exposure)
Possible exposure to toxins including drugs (e.g., an anthelmintic with a known teratogenic effect)
Environmental factors (e.g., extreme heat during early pregnancy, stress, predation)
Clinical illness in the individual ewe either before, during, or after abortion

SPECIMENS TO SUBMIT TO DIAGNOSTIC LABORATORY

Fetus and Placenta

- Gloves and protective clothing should be worn for handling fetuses, placentas, or discharges.
- All fetuses and placentas that can be obtained should be submitted for analysis.
- Contaminants such as manure or dirt should be gently removed from the specimens.
- The specimens must be kept chilled (NEVER frozen!).
- Specimens are submitted in leakproof, clean containers (e.g., no feed bags) as quickly as possible and kept chilled.

Processed Samples

If swabs, fetuses, and placentas cannot be sent for evaluation immediately, then the farmer should contact the flock veterinarian to collect appropriate samples of the fetuses and placentas.

Serum from Ewes

- Specimens from all aborting ewes and from a minimum of 10% of pregnant ewes should be obtained.
- Paired sera—acute and convalescent (10 to 21 days after acute sample)—are submitted to demonstrate a rising titer.

MANAGEMENT OF THE ABORTING FLOCK

Management should begin with removal of pregnant ewes from the aborted ewe group to a clean area. Mixing of pregnant, at-risk ewes with other groups of livestock should be prevented. Aborted ewes should be left in the contaminated pen or pasture.

Specific control measures may be initiated on the basis of a likely diagnosis, before diagnosis is confirmed.

Some of the infectious agents responsible for abortion in sheep pose significant zoonotic risks. The farmer should be advised to wear gloves, boots, and protective clothing that are changed before managing the rest of the flock. The unaffected sheep should be examined first. Filter masks should be worn for cleaning the barn. Pregnant women should not assist at lambing and should, if possible, not have contact with the pregnant, aborting, or newly lambed sheep and lambs.

If the aborted ewes are to be culled, they should be sent directly to slaughter once the vaginal discharge has cleared. They should *not* be sold at an auction, where they might be taken into another flock as breeding animals.

INFECTIOUS CAUSES OF ABORTION

Viral Causes

Bluetongue

Agent. Bluetongue virus (BTV) is an arthropod-transmitted orbiviral disease of domestic and wild ruminants. In North America, it is found endemically in the southern and western United States, as well as in parts of the Caribbean, Mexico, and Central America.[1] Serotypes known to occur in these regions include 10, 11, 13, and 17. In Canada, occasionally serologic reactors (mostly bovine) to serotype 11 have been identified in the Okanagan Valley of British Columbia.[2] At least 24 serotypes have been recognized worldwide.

Transmission. The virus requires an arthropod midge vector, *Culicoides veriipennis,* for transmission. The subspecies *C. veriipennis sonorensis,* found mostly in the Southwest, is thought to be the most important vector. Clinical expression of the disease tends to be seasonal (fall) and linked to the midge's seasonal cycle. Preferred breeding conditions include predominantly wet conditions and a warm environment. Cattle—considered to be an important vertebrate reservoir—can remain viremic for up to 90 days and show no clinical signs. Infected sheep clear the virus within 21 days but may experience a case fatality rate of 10% to 50%. BTV also has been isolated from bovine semen. Although the vector is found across southern Canada, whether it can propagate infection is questionable.

Clinical picture. Sheep infected with BTV show signs of high fever; swollen ears, face, and tongue; oral and nasal ulcers; and lameness. Up to 20% of fetuses infected in early gestation are found to have various degrees of hydranencephaly and skeletal deformities and may be aborted or carried to term. Some experimental studies have failed to show disease in fetuses born to infected ewes.[3] Plant toxicity and Cache Valley virus (CVV) infection are important to rule out in the diagnosis.

Diagnosis and control. Some areas of the United States (e.g., California) may have serotype-specific attenuated virus vaccines available. Little to no cross-protection occurs between serotypes. Sheep need to be vaccinated once before the season of risk and again before breeding to avoid congenital central nervous system (CNS) lesions.[4,5] Control measures also can include housing sheep at night and/or pasturing away from wet areas during the season of peak vector activity. In Canada, suspected cases of bluetongue must be reported to the local office of the Canadian Food Inspection Agency. Virus isolation and serologic studies in the aborting dam are done to rule out infection with BTV.

Border Disease

Agent. Border disease virus (BDV) is a pestivirus closely related to bovine viral diarrhea virus (BVDV) and classical swine fever virus (CSFV). It has been reported sporadically across North America and in many parts of the world. Many different strains of BDV have been isolated from animals with clinical border disease.[6] Some outbreaks of border disease have been associated with persistent infection with BVDV.[7]

Transmission and pathogenesis. The virus invades the ewe and causes a transient viremia of 7 days' duration; it does not cause disease in the ewe but attacks the placenta. During the viremia, BDV is shed in the urine, feces, and saliva. Necrosis occurs at the junction of the villous trophoblast and maternal caruncle, with resultant perivascular necrosis. This necrosis leads to separation of the fetal-maternal-placental bond, with subsequent interference with fetal nutrition and blood gas exchange.[8] Most fetuses infected in utero before day 60 to 85 of gestation die because of this damage and are either reabsorbed, aborted, macerated, or mummified.[9] If they survive, various degrees of damage may be seen. Myelination of nerve cells is disturbed, particularly in the cerebellum. Hair follicles also can be affected and produce hair, rather than wool. These fetuses also are born persistently infected with BDV, shed virus continuously, and fail to clear the infection. Fetuses infected after day 85 may abort, or the lamb may be born weak or unaffected, or may be virus negative and with a precolostral titer to BDV. It is possible for several levels of signs to be present in the same litter of lambs.

Clinical picture. An increase in the number of open ewes, as well as abortion, is seen in the flock, followed by the birth of lambs with or without clinical signs, including weak lambs that are persistently infected.[6] These lambs are small, with shortened facial and long bones, and have a wool coat that frequently is hairy and darkly pigmented, particularly over the shoulders, neck, and head. They also may show mild to severe body tremors. Accordingly, affected lambs commonly are termed "hairy shakers." Nervous signs diminish in severity in those lambs that survive. Persistently infected lambs grow more slowly than do their healthy cohorts and appear to be more susceptible to disease.[10] Some evidence indicates that persistently infected lambs may succumb to a disease similar to mucosal disease of cattle.[11] At necropsy, findings include gross thickening of the distal ileum, cecum, and colon resulting from focal hyperplastic enteropathy, and cytopathic BDV can be recovered from the gut.[6] Usually a history of a recent introduction of animals either before, during, or shortly after breeding season can be elicited from the farmer, or the disease may follow contact with cattle, suggesting exposure to BVDV.

Diagnosis. BDV can be isolated from aborted fetuses and from the buffy coat in centrifuged blood specimens

from affected lambs. Antigen-capture enzyme-linked immunosorbent assay (ELISA) can be used on the sera of suspected persistently infected adults and lambs older than 2 months.[6,12] CNS changes, both gross (cerebellar hypoplasia, hydranencephaly) and microscopic (hypomyelination, microgliosis), help make the diagnosis. Immunohistochemical analysis is equally sensitive to virus isolation, except in autolytic fetuses.[13] In autolytic fetuses, use of formalin-fixed, paraffin-embedded fetal brain for immunohistochemical analysis is more sensitive than virus isolation. It also appears to be more sensitive than antigen-capture ELISA on fetal tissues.[13] Serum virus neutralization or ELISA titers of antibody from the aborted ewes tend to be high, although this finding in itself is not diagnostic. Demonstration of a rising titer in aborting ewes is difficult because infection generally is historical—preceding abortion by at least 2 months. If an aborting ewe is persistently infected, then antibody levels will be low or absent and virus can be isolated or detected from the serum or buffy coat.

Control and prevention. After an outbreak of border disease–related abortion, all lambs with congenital pathologic conditions and those demonstrating poor performance should be sent to slaughter when they are as young as possible and, before that, should be kept separate from the breeding flock. Replacement lambs older than 2 months should be screened for virus using virus isolation or antigen capture ELISA.[6] New additions to the flock should be isolated for at least 2 weeks before introduction to the breeding flock, and consideration should be given to screening them as well. Cattle and sheep should be kept well separated, with no shared feeders or water sources, and an effective vaccination program for BVDV should be maintained in the cattle herd. Available evidence suggests that cross-protection is poor between BVDV and BDV; therefore, vaccinating sheep with a killed or modified live BVDV vaccine confers no advantage.[6] At present, one border disease vaccine is commercially available.[14] It is a killed adjuvanted vaccine that contains strains of BDV as well as of BVD-1.

Cache Valley Disease

The agent. The Bunyaviridae family, of which CVV is one species, contains several species that are associated with ovine abortion. Organisms in this family of viruses are arthropod borne. Akabane virus is considered to be exotic to North America but is a very important cause of congenital ruminant deformities in Australia, Japan, Korea, and Israel and probably also is present in the Middle East and Asia. In North America, CVV has been implicated in outbreaks of congenital deformities in the U.S. Southwest, particularly Texas, and occasionally in the Northeast (Illinois, Michigan, New York, Maryland, and Pennsylvania) and southern Ontario.[15] Experimentally, Main Drain virus and, to a lesser extent, San Angelo and LaCrosse viruses also cause ovine fetal malformations.[16] LaCrosse virus is an important cause of viral encephalitis in children.[16]

Transmission and pathogenesis. CVV is transmitted mainly by mosquitoes that prefer to feed on large ruminants. Akabane virus mostly is transmitted by *Culicoides* midges. The vertebrate reservoir for CVV is not known but is speculated to be wild or domestic large ruminants (e.g., deer, cattle). Two days after infection of the sheep by the infected mosquitoes, a viremia of 2 to 5 days' duration develops. At this time the infected sheep may demonstrate fever, depression, and a reluctance to move. The virus invades the placenta and fetus if implantation has occurred. Little inflammatory response is observed in the placental tissues. The virus then invades the fetal brain, spinal cord, and skeletal muscles. The severity of the lesions is dependent on gestational age at the time of infection. Early infection from day 28 to 32 generally results in early embryonic death and mummification. Infection between days 32 and 37 manifests as CNS and musculoskeletal deformities characteristic of severe arthrogryposis. Infection from day 37 to 48 manifests as milder musculoskeletal lesions with nonsuppurative encephalitis and encephalomyelitis.

Clinical picture. The dominant clinical picture is one of stillbirth or live birth of congenitally deformed lambs. The predominant deformities are arthrogryposis, brachygnathia, hydranencephaly/porencephaly, microencephaly, agenesis or hypoplasia of the spinal cord, and possibly other CNS deformities. It is possible to see all syndromes in one flock outbreak and more than one syndrome in a litter. Morbidity rates are variable depending on viral load in the mosquito population. Fetuses from cattle and goats also may be affected.

Diagnosis. It is important to rule out ingestion of locoweed as a cause for arthrogryposis. Osteochondrodysplasia (spider lamb) is inherited in an autosomal recessive fashion and affects mainly Suffolks and Hampshire sheep; neurologic disease and arthrogryposis are not seen. By the time of parturition, CVV has been eliminated from the dam and fetuses, so that virus isolation is unrewarding. In endemic areas, ewes may have background titers to the virus. Diagnostic titers can be obtained only from fetal heart blood or precolostral serum from affected lambs. Absence of a diagnostic titer in the fetus or lamb will not rule out CVV infection but in the dam does rule out this disease.

Control and prevention. No vaccine is available to prevent CVV in sheep, although an inactivated vaccine for Akabane virus is available in Australia and parts of Asia. Immunity from natural infection is long term and may be for life. It is difficult to predict an epizootic of CVV infection. In geographic areas with a history of CVV infection, the farmer is advised to avoid breeding sheep in mosquito-infested pastures; in temperate parts of North America, the farmer should wait until after a killing frost before turning in the ram.

Bacterial/Chlamydial/Rickettsial Causes

Brucellosis

Agent. *Brucella ovis* is a common cause of epididymitis in rams and more rarely causes abortion in ewes. It is widely distributed in the United States and to a lesser extent in Canada, and more commonly in the western regions. *Brucella melitensis* also can cause ovine abortion and is found in Mexico but not in the United States or Canada.[17]

Transmission and pathogenesis. The organism is spread through contact between mucous membranes (vaginal, preputial, conjunctival), both ram to ewe and ram to ram, but not ewe to ewe. Rams infected with this organism are not always infertile and pass the organism in the ejaculate. Ewes clear the organism within a few weeks of aborting and usually are not sources of infection to the flock the following year. Sources of infection may be the carrier ram and infected material from abortions.

Clinical picture. Abortion levels of 25% to 35% have been reported. Stillbirths and lamb weakness also may be seen; affected animals may show lesions of suppurative pneumonia.[18] Placentitis is severe, with a thickened, necrotic placenta—a common finding. Examination of the breeding rams will reveal clinical or subclinical epididymitis.

Diagnosis. Titers in the sera of aborting ewes are variable, and such tests may be misleading because a negative titer does not rule out the disease. The sensitivity of the complement fixation test ranges from 96.3% to 100% and the specificity from 98.4% to 99.9%. The ELISA specificity is reported at 99.4% and the sensitivity at 100%.[19] Culture of or use of a polymerase chain reaction (PCR) assay on the stomach contents of the fetus, as well as culture of the cotyledons, generally is successful.[20] Serologic testing and examination of the rams also should be performed. *B. melitensis* infection should be a consideration in the differential diagnosis, particularly in states bordering Mexico.

Prevention and control. In endemic areas, such as the western part of North America, a control program using a combination of serologic testing (ELISA or complement fixation test or both), routine scrotal palpation of breeding rams, and vaccination of ram lambs with a killed bacterin will reduce the prevalence of disease.[21] The vaccine is regarded as having poor efficacy, and the serologic tests and scrotal palpation have only fair sensitivity. Rams should not be shared between flocks, nor should they be housed at any time with rams used in other flocks. In Canada, serologic testing is not readily available for screening of rams. Rams from affected flocks should be slaughtered and replaced with virgin rams from clean sources.

Vibriosis/Campylobacteriosis

The agent. Both *Campylobacter jejuni* and *Campylobacter fetus* subsp. *fetus* have been implicated in ovine abortion, and both are also pathogenic to humans. They can reside in the intestinal tract of sheep and many other species, including dogs and birds. *C. jejuni* is implicated more commonly when abortion is sporadic, although it can affect up to 20% of the pregnant flock. *C fetus* subsp. *fetus* is more frequently associated with large outbreaks of abortion that affect a significant percentage of the flock and may occur from year to year. Several serotypes of *C fetus* subsp. *fetus* have been recognized. In the United States, most of the isolates associated with abortion are A2 and C.[22] Occasionally other species and biovars of *Campylobacter* have been isolated from aborted ovine fetuses.

Transmission and pathogenesis. The organisms are harbored in carrier sheep in the intestine and gallbladder.[23] Sources of contamination are feces, aborted fetuses and placentas, and vaginal discharges from aborted ewes. Carrion-eating birds such as crows may transmit the organisms between flocks. Incubation ranges from 8 to 60 days.[24]

Clinical picture. Ewes generally start to abort in the second half of gestation. Most abortions occur during the third trimester about 3 days after fetal death.[25] Fetuses are expelled well preserved with the placenta. Ewes are not ill, although transient diarrhea has been reported. Ewes infected 2 weeks before lambing may give birth to stillborn lambs, or to weak lambs with a very poor chance of survival. The placenta of aborted fetuses tends to be edematous, with congested swollen cotyledons. The fetus may have subcutaneous edema and an enlarged abdomen with pleuritis, peritonitis, and hepatitis, with occasional target lesions of hepatic necrosis. Abortion levels of up to 70% have been reported, but levels of 10% to 20% are common in enzootically infected flocks. Immunity from one species of *Campylobacter* species is not cross-protective for another, but natural immunity to the infecting species can occur without abortion and lasts at least 3 years.[26]

Diagnosis. Stain impression smears of cotyledons or fetal stomach contents will demonstrate the organism. Darkfield or contrast microscopy will demonstrate the live organism from fetal stomach contents. It also can be cultured from the placenta or stomach contents. *Flexispira rappini* infection should be a consideration in the differential diagnosis.[27] Antibiotic sensitivity testing should be done on the isolate, because resistance to penicillins and tetracyclines can occur.[28]

Prevention and control. In the face of an outbreak, all pregnant ewes should receive treatment with an antibiotic that is likely to be efficacious. Long-acting oxytetracycline at the label dose (20 mg/kg) can be used successfully. For large flocks, for which individual injections may be more difficult, tetracyclines may be added to the feed at a level of 250 to 300 mg/head/day until lambing is finished.[29] Daily injections of penicillin (400,000 IU) and dihydrostreptomycin (0.25 g) at a dose rate of 6 to 10 ml per ewe also have been recommended.[30] Pregnant ewes should be removed from the aborted ewe flock and the contaminated area. Vaccination with a multivalent bacterin can be done in the face of an outbreak, but sufficient immunity to stop abortions takes 2 weeks to develop.[29] In flocks in which campylobacteriosis has been diagnosed, vaccination should be routinely done according to label directions. This protocol involves vaccinating all breeding ewes with a bivalent killed bacterin twice, the first before breeding and the second injection 60 to 90 days later. Annual revaccination before breeding should be maintained.

A lack of efficacy of the vaccine may be due to any of several factors. The vaccine may not contain the correct serotype of *C. fetus* subsp. *fetus,* or the responsible pathogen may be another species of *Campylobacter* or *F. rappini.*[22] Culture and typing should be done to rule out this possibility.

Enzootic Abortion of Ewes (Chlamydiosis)

Agent. The genus *Chlamydia* has recently undergone reclassification.[31] *Chlamydia psittaci* immunotype 1, the abortion strain for sheep and goats, is now classified as *Chlamydophila abortus* type strain ATCC VR 656. *Chlamydia pecorum,* a fecal chlamydial species that may be nonpathogenic or cause arthritis and conjunctivitis and often cross-reacts on tests with *Chlamydia abortus,* is now classified as *Chlamydophila pecorum* type strain ATCC VR 628, Bo/E58. Organisms belonging to this genus are obligate intracellular bacteria that replicate in the phagosome.[32]

Transmission and pathogenesis. Transmission may occur from exposure to aborted materials or vaginal discharge or from environmental contamination and ingestion.[32] Experimental oral inoculation of the tonsillar crypts, but not intraruminal inoculation, produces abortion.[32] Contact of the nasopharynx with infected placentas in recently lambed ewes can cause abortion in those ewes during the subsequent pregnancy.[33] *Chlamydia*-naive pregnant sheep infected vaginally will give birth to infected weak lambs, suggesting that the venereal route also may be important in the transmission of this agent.[34] When naive nonpregnant sheep were experimentally infected, the organism could be detected in a variety of organs, including the abomasum and jejunum, and lymph nodes, but shedding in the feces was not detected.[35] *C. pecorum* strains, frequently present in large numbers in the feces of healthy ewes, can cause abortion when injected into the muscle of healthy ewes, but not when ingested.[32] In nonpregnant ewes, at approximately 1 week after infection, *C. abortus* becomes undetectable by any means.[36] In infected rams, *C. abortus* may be isolated from the semen and seminal vesicles.[36] When the infected ewe becomes pregnant, the organism moves by a hematogenous route to the trophoblast cells of the chorionic epithelium.[36] By 95 days of gestation, the infection has spread from the cotyledons to the intercotyledonary areas.[39] The fetus also becomes infected, but histopathologic changes are minor. The primary target is the placenta.

Ewes rarely abort at less than 100 days' gestation, but fetal loss and resorption before 100 days can occur.[34] Latent infections occur when ewes are infected either while not pregnant or during late gestation. These ewes will abort during the subsequent pregnancy.[32] Ewes that abort once do not abort again for at least 3 years. Although maternal immunity prevents repeated abortion, ewes that have aborted from *C. abortus* infection subsequently shed the organism in vaginal secretions during estrus and have persistent low-level titers.[37] Contamination of mucous membranes or of feed by vaginal secretions, or of the ram's penis during breeding, may be a mechanism of continuing the infection in the flock.

Clinical picture. Abortion tends to occur in late term pregnancies, but early fetal death and resorption also occur.[37] The placenta is necrotic, with lesions affecting both cotyledons and intercotyledonary spaces. The placenta becomes thickened and necrotic, as well as hemorrhagic at the edges of the lesions.[39] Fetuses may be aborted necrotic, well preserved, or rarely mummified. Weak and stillborn lambs also are commonly seen. Abortion levels can be high (up to 30%) in the first year of the disease but decrease in subsequent years to 10% to 15%, with only ewe lambs and new introductions affected in subsequent years. Ewes infected in very late pregnancy or when open may not develop protective antibodies and may be susceptible to infection in the next pregnancy. Chronic infection of the uterus or uterine tubes occurs after abortion and may eventually impair fertility.[34]

Diagnosis. The severe placentitis of both the cotyledons and intercotyledonary zones is typical but not diagnostic. Smears made from the chorionic villi and stained with appropriate stains (e.g., Machiavello, Wright-Giemsa, xanthine dye–thiazine dye mixture, Giminez, modified Ziehl-Neelsen, or *Brucella* differential) will demonstrate clumps of small, red, coccoid intracellular elementary bodies.[40] The organisms can be confused with *C. burnetii*. Immunologic staining methods such as those using immunoperoxidase can be used on fixed tissues.[41] Various tissues (fetal lung, spleen, liver, placenta, and vaginal swabs) also can be submitted for culture using either a *Chlamydia* isolation procedure modified from Stortz or embryonated chick eggs.[40] Chlamydiae are difficult to isolate, however, because they lose infectivity during transport to the laboratory.[39] Fluorescent antibody can be used to detect the organisms in smears and culture, but this technique appears to be less sensitive but perhaps more specific than a chlamydial genus–specific ELISA that detects lipopolysaccharide.[42]

Infected ewes that have not yet aborted do not have significantly elevated antibody titers. Serologic testing of aborting ewes should always use paired acute and convalescent sera. Low-level titers detected in screening the breeding flock may be due to a lack of test specificity, or to chronic *C. abortus* infection.

The complement fixation test is not very specific because it detects genus-specific lipopolysaccharide, so that cross-reaction occurs with other species of *Chlamydophila,* notably *C. pecorum*.[34] This test should not be used for diagnosis in individual aborting sheep or in lambs or rams, or beyond 3 to 6 weeks after abortion.[32] Many ELISAs that detect lipopolysaccharide or outer membrane or unstated antigens failed to perform adequately with respect to either specificity (range of 51% to 96%, depending on the test), sensitivity (range of 51% to 81%, depending on the test), or both.[43] A new indirect ELISA based on detection of the outer membrane protein of *C. abortus* has improved sensitivity (84.2%) and specificity (98.5%) over the complement fixation test.[44] PCR assay is highly sensitive, but again specificity may be poor owing to the contaminating presence of *C. pecorum*.

Prevention and control. At present, only inactivated vaccines produced from cultures of *C. abortus* are available in North America. The vaccination procedure is to give an initial injection 60 days before breeding and administer a second dose 30 days later. Annual revaccination is required. Inactivated vaccines do not prevent shedding of chlamydiae at lambing.[32] Prolonged use of these vaccines may even select for more virulent field

strains of *C. abortus*.[32] Inactivated vaccines have been shown to provide reasonable protection,[45] but a potential problem is considerable batch-to-batch variability, with variable potency of the vaccine, which may account for failure of some vaccines to elicit an immune response or to provide protection.[32,46] For this reason, the use of a temperature-sensitive mutant of *C. abortus* (1B strain) as a vaccine was investigated. The result was the production of a commercial vaccine (Enzovax*), which is not available in North America. When injected in ewes or does before breeding, it did not interfere with the subsequent gestation, it protected against abortion, and it prevented chlamydial shedding at delivery.[32] Vaccination of an uninfected flock will offer good protection if the disease is acquired after vaccination. If vaccination is not done until the disease has been diagnosed in aborting ewes, the subsequent lambing will still see significant levels of abortion due to latent infections. After the second year of flock vaccination, however, the abortion level in infected flocks will decline, because fewer susceptible animals exist. To increase ease of use, research also has been looking at developing an effective vaccine using purified subcellular antigens such as the outer membrane protein (90 kDa), which produces a very strong protective immune response after abortion.[47]

In an outbreak or commencing after 80 days of gestation in flocks known to be infected, injections of long-acting oxytetracycline at label dose (20 mg/kg of body weight given once), and repeated every 2 to 3 weeks, may prevent some abortions, although probably less than 50%, and some authorities disagree whether use of multiple treatments is justified.[48–50] Often the placental damage in pregnant ewes is so severe that antimicrobial therapy is of limited efficacy, and shedding of organisms at lambing is not reduced.[48,49] Pregnant ewes should be separated from the aborted ewes, and ewe lambs should be managed as a separate flock until after lambing. In view of the persistence of the organism in vaginal secretions, culling of ewes that have aborted should be considered. In chronically infected flocks, feeding levels of 250 to 500 mg/animal/day of tetracycline, starting 60 days before the first expected lambing date, is thought to be helpful in reducing the incidence of abortion (M. Bulgin, personal communication, 1998). Higher levels generally are fed during an outbreak of abortion, and lower levels are fed prophylactically, although actual recommendations on dose and length of time vary, and little information on true efficacy has been published.

Eradication of *C. abortus* from an infected flock is difficult. In the U.K., accreditation of *Chlamydia* status is based on serologic status of the breeding flock. Test sensitivity and specificity currently hamper success of these kinds of programs. Embryos obtained from infected ewes do not appear to harbor the organism, however, and can be used to transfer valuable genetics to a clean flock.[51]

Zoonotic risk. Occasionally *C. abortus* can cause significant human disease, particularly in pregnant women with significant contact with aborting or lambing ewes.[52] Infection may be more common than disease, but because of the risk of fetal or maternal death, it is important that producers and veterinarians handling infected animals take appropriate precautions.[53]

*Intervet, Milton Keynes, UK.

Coxiellosis (Q Fever)

Agent. *Coxiella burnetii* is a rickettsial organism (an obligate intracellular gram-negative bacterium) that can infect a wide range of hosts, including ruminants (cattle, sheep, and goats), swine, guinea pigs, cats, dogs, rabbits, rodents, and humans, as well as birds and ticks.[54–59] It can survive in a dried state for many years.

Transmission and pathogenesis. Inhalation of contaminated air or dust, or mucous membrane contact with aborted materials, vaginal secretions, or fluids and membranes from normal birthing of many species, as well as possibly semen from infected males, can serve as a means of acquiring infection.[60] Ticks also may shed the organism, thereby contaminating the wool. If the ewe is pregnant and immunologically naive, then placentitis may develop; if this infection is severe enough, abortion due to hypoxia and starvation of the fetus may result. Abortion is more common in goats than in sheep. Although the organism commonly is shed in the milk of cattle and goats, it does not appear to be shed in the milk of sheep.

Clinical picture. In sheep, infection at lambing is more commonly seen than abortion. Abortion rates within a flock can range from 5% to 35%. Fetuses may be aborted in late term, and in the same outbreak, lambs may be stillborn or born weak; however, severe suppurative placentitis is a common feature with all three presentations. Uterine inertia and uterine rupture at the time of abortion have been reported in goats.[61] Abortion in subsequent years is unusual because most of the flock is exposed and immune. The risk is that the fluids and the newborn lamb from a healthy lambing constitute a source of infection to humans. In a flock with little abortion, as many as 47% of the lambing ewes may be shedding *C. burnetii* in the vaginal secretions for up to 2 months after lambing, and significant proportions also shed the organism in the milk (18%) and feces (6%) during the first week after lambing.[62] It is common to have no reports of abortion despite endemic infection in the flock.[63]

Diagnosis. Either demonstration by staining with a modified Ziehl-Nielseen stain or culture of the organism from the cotyledons and fetal abomasal contents is a highly reliable method of diagnosis. Histologic examination of the placenta reveals a foamy vacuolation of the trophoblast cells of the chorion.[61] Analysis of paired sera using a complement fixation test or ELISA will demonstrate very high titers in some flocks; this finding, however, is not diagnostic for abortion but rather is indicative of active or recent infection only. During infection, two distinct antigenic phases are recognized. Phase 1 antibodies are produced during the acute infection stage. Phase II antibodies are produced later in the disease process or are produced more prominently by some strains of *C. burnetii*. Only moderate correlation has been found between ELISA titers and shedding.[62] Several PCR tests have been developed to detect shedding in the milk, feces, and vaginal secretions.[64,65]

Prevention and control. No commercial vaccines for livestock are available in North America, although killed vaccines are available in Europe. The best protection appears to be offered from chloroform-methanol residue vaccines made from phase 1 organisms, although they may not stop shedding.[59,66] In the face of an outbreak, long-acting oxytetracycline injections at 20 mg/kg have proved effective in stopping abortions but do not clear the carrier state.[62] Cats, cattle, goats, and rodents also may be a source for continuing re-infection. Moreover, the organism can remain viable in a dried state in the environment for years, which also can lead to re-infection. It is doubtful if eradication of the organism can be accomplished on a commercial farm but may be possible in a research institution when strict culling and disinfection are practiced.

Zoonotic disease. The disease in humans—Q fever—makes this organism very important.[67] Although most people who become infected do not become critically ill, the risk of severe and prolonged illness is significant. Disease in humans can manifest as acute atypical pneumonia, undulant fever, hepatitis, extreme myalgia, or endocarditis, which is the most common form of chronic Q fever.[59] Prompt diagnosis and treatment are necessary for full recovery. Physicians generally are unfamiliar with the disease, and the vagueness of the signs may delay appropriate therapy. Death is a risk, particularly for elderly and immunocompromised patients. People may become infected from handling infected placentas and lambs, from being present when sheep are lambing, and from inhaling windborne organisms from infected premises or dried organisms in the dust of barns.[68–70] In one outbreak associated with goats, the most important risk factors were contact with the placenta (odds ratio [OR] 12.32), being a smoker (probably increases the risk of oral contamination, OR 3.27), eating goat cheese (OR 5.27), and petting goats (OR 4.33).[71] Unsuspecting persons may become infected at shows or in sales barns where dust is evident. Barn clothing should never be worn in the house.

Disposable rectal sleeves should be worn by persons assisting with lambing or handling an abortus or the placenta. Masks should be worn for cleaning the barn. Vaccination of humans at risk for contracting Q fever should be practiced. Abattoir workers in Australia are routinely vaccinated.[59] In view of the difficulty of eradicating coxiellosis from domestic livestock and preventing shedding in infected animals, vaccination of farm workers and researchers offers a means to decrease human risk.

Parasitic Causes

Toxoplasma gondii *Infection*

Agent. *Toxoplasma gondii* is a protozoal parasite that has a worldwide distribution. In many countries, it is one of the most commonly diagnosed causes of ovine abortion. The sexual part of the organism's life cycle is completed only in domestic and wild cats. The oocysts shed in their feces are infective for up to 18 months when protected from desiccation and sunlight. The asexual component of the *Toxoplasma* life cycle may occur in any warm-blooded animal.[72]

Transmission and pathogenesis. Nonimmune cats, particularly kittens first learning to hunt, may become infected by ingesting food or animals containing cysts, including rodents, offal from slaughtered farm animals, birds, and aborted fetuses and placentas. Cats rarely show clinical illness and will shed millions of oocysts from 4 to 12 days after ingestion of contaminated material and then become immune, although they may again excrete oocysts in smaller numbers if stressed.[72] They also may be infected by ingesting sporulated oocysts shed by other cats, although this results in fewer numbers being shed and a longer incubation period.[72] Sporulation occurs within 4 days of excretion. Feces and thus oocysts are ingested by rodents or inadvertently by sheep through contamination of feed or pasture. The organisms invade by passing through the small intestine to the mesenteric lymph nodes, and a parasitemia develops, which generally lasts 5 to 12 days after infection.[72] As the animal develops immunity, the tachyzoites encyst in the muscle and brain as bradyzoites. They persist throughout the life of the animal and can be infective after death in chilled carcasses for up to 60 days. In a pregnant sheep, approximately 14 days after ingestion, the organism infects the fetus through the trophoblast cells of the fetal villi.[72]

Clinical picture. Fetuses at all stages of gestation are susceptible to infection, although the clinical picture varies. Infection before 40 days results in resorption; between 40 and 120 days, in maceration, mummification, or abortion; and after 120 days, in stillbirth or birth of weak or healthy immune lambs. Abortion levels within the flock may vary, ranging from 5% to 100%. Ewe immunity, timing of exposure, and dose of oocysts determine the abortion rate. The flock history usually includes contact with kittens, either directly or from fecally contaminated forages or grain. A few weeks to days before the expected onset of lambing, ewes may start to abort. Often all levels of infection can be observed within the same outbreak and sometimes within the same litter—for example, a weak lamb may be born along with a mummified fetus.[72] Liveborn weak lambs often do not survive beyond the first week. Ewes do not appear ill and recover from the abortion quickly. Immunity to further abortion from toxoplasmosis appears to be life-long, although this may not be true in goats. In a study done on Ontario farms that examined the proportion of ewes that were seropositive, spaying female cats and shearing the perineum of the ewe before lambing were protective, whereas sharing pasture with other livestock species, indoor summer housing of replacement ewes, purchasing sheep from other flocks, and raising pigs on the same farm all were found to increase the prevalence of *Toxoplasma*.[73]

Diagnosis. The fetuses may be mummified or aborted in a decomposed state. Subcutaneous edema and blood-tinged fluid with strands of fibrin in the body cavities may be seen. The placental cotyledons appear bright to dark red and speckled with white foci of necrosis 2 to 2 mm in size.[72] The intercotyledonary placenta appears normal. Histologic examination of these necrotic foci also may reveal calcification. The toxoplasmas are not numerous and often are seen only on the periphery of lesions.

Because of the marked decomposition usually present in the aborted fetus and placenta, however, the organism is not easy to discern on histopathologic examination. Immunohistochemical techniques (either an indirect immunoperoxidase or a peroxidase-antiperoxidase method) will help demonstrate organisms more clearly, even in decomposed tissues.[72,74] PCR assay also can be used successfully to detect the presence of organisms in decomposed placentas.[75–77]

Serologic studies may be done using either an immunofluorescence antibody test or an ELISA or latex agglutination test.[78,79] Approximately 2 weeks after infection, titers rise and remain high for several years.[80] A suggested interpretation of latex agglutination test titers is as follows: less than 32 indicates a negative result; 32 indicates a weak positive or nonspecific result; a titer of 64 to 256 indicates a positive result but possibly declining or long-term static; and a titer of 512 or greater indicates a positive result and possibly is indicative of a recent or active infection.[79] A seropositive flock is one with at least two ewes with titers of 128 or greater. A positive titer is not necessarily an indication of abortion, because titers as high as 2048 have persisted for up to 3 years in some ewes.[79] A positive titer from an aborted fetus is diagnostic. Fetuses infected between 45 and 90 days of gestation have been shown to seroconvert within 28 to 35 days after infection.[81] Negative titers in the ewe will aid in ruling out toxoplasmosis.

Control and prevention. In flocks in which toxoplasmosis has been diagnosed, little can be done during that lambing period. To prevent infection from being continued to the following year, contaminated bedding should not be spread onto pastures the following year.[82] Cats should be prevented from defecating in feed or bedding by either eliminating all farm cats, spaying all queens to reduce the population of kittens, or preventing access of cats to sheep feed. Contamination of stored feeds can be reduced by sealing grain in metal bins (with the added benefit that rodents cannot get access either), by providing a cat litter box in the barn to discourage defecating in feeders, and by not feeding square bales stored on top to pregnant ewes, because they may be contaminated by cat feces. These bales can be put aside and fed later to lactating ewes. Water sources should be raised so as to reduce the risk of contamination with feces. These control measures may still be inadequate, because a significant feral cat population usually exists in most rural areas and infection can occur on pasture where kittens are learning to hunt. Commercially prepared supplements may be contaminated without the producer's knowledge. In addition, mice can transmit the infection vertically to their fetuses, thereby perpetuating a reservoir of infection for new generations of kittens.[83]

Feeding monensin (Rumensin, Elanco) throughout gestation at a dose rate of 15 mg/head/day throughout pregnancy has been shown to be an effective prophylactic measure.[83] Decoquinate (Deccox*), if fed at a rate of 2 mg/kg of body weight daily throughout gestation, also protects against infection.[84] Prophylactic use of chemotherapeutics should be considered if toxoplasmosis persists in the flock despite use of the preceding control measures.

Using live tachyzoites of the S48 "incomplete" strain of *T. gondii*, a vaccine (Toxovax*) has been developed that confers excellent immunity for at least 18 months and probably for life but is not yet available in North America.[85] Inactivated tachyzoite vaccines have failed to provide protection against abortion, even if the vaccine elicited a good humoral response.[85] The S48 vaccine is given at least 3 weeks before mating to the entire female breeding flock and, in subsequent years, only to female flock additions. The drawback with this the vaccine is that it has a very short shelf life (7 to 10 days) and is capable of infecting humans.

Zoonotic disease. Approximately 30% of adults in the United States have antibody titers to *T. gondii*.[86] Pregnant nonimmune women are the most at risk of disease. *T. gondii* can cause congenital neurologic disease and blindness in human fetuses. It is an important cause of encephalitis in humans suffering from acquired immunodeficiency syndrome (AIDS). Most humans probably become infected from consuming undercooked meat, although handling of cat feces should be considered as a source. Freezing meat to –12° C for 1 day or cooking meat to 67° C will kill tissue cysts.[86] Microwave cooking is uneven and may leave some cysts viable.[86]

Miscellaneous Infectious Causes

Endotoxin Abortion

Available evidence indicates that in many species, endotoxins released from gram-negative bacteria (e.g., coliform mastitis, salmonellosis, contaminated drugs, sepsis) cause release of prostaglandins in very high levels, with subsequent abortion. The abortifacient effect during pregnancy in sheep has not been well studied, however.[87,88]

Flexispira rappini *Infection*

The clinical presentation of abortion following infection due to *F. rappini*, a flagellated, anaerobic agent, is very similar to that of abortion following infection due to *Campylobacter* spp.[27,89] Suppurative placentitis and fetal liver necrosis are features, as well as mummified fetuses, abortions, and stillbirths. Findings on darkfield microscopic examination of fetal stomach contents are negative, however, in cases due to *F. rappini*. Culture of liver, stomach contents, and placenta on blood agar, incubated in an atmosphere of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide, will grow filamentous organisms that stain faintly gram negative.[89] The organism is sensitive to oxytetracycline.

Histophilus ovis *Infection*

Histophilus ovis has been implicated in ovine mastitis, polyarthritis in lambs, epididymitis of rams, and many other pathologic conditions. It also is occasionally a cause of ovine abortion.[90] The organism not unusually is found

*Alpharma, Fort Lee, NJ.

*Intervet, Milton Keynes, UK.

in vaginal cultures of young ewes. It is speculated that abortion is caused when the organism ascends in carrier ewes.[90,91] Abortion may be sporadic or occur in up to 50% of pregnant ewes. The placenta is hemorrhagic and swollen. The fetuses may be mummified or necrotic. Diagnosis is made by culture of the fetus or placenta. Control should focus on the entire flock, because the organism may be present in rams or carrier ewes.

Leptospirosis

The most common agent of leptospirosis in sheep is *Leptospira interrogans* serovar *hardjo,* although *Leptospira pomona* has been isolated in some cases.[92] It is an unusual cause of abortion in sheep but should be considered in areas in which leptospirosis is a problem in cattle, pigs, or wildlife. Ewes abort or have stillborn lambs and also may have high fevers, flaccid agalactia, hemoglobinuria, and jaundice. An important consideration in the differential diagnosis for *Leptospira*-related abortion is copper toxicosis.

Diagnosis is confirmed by a fourfold rise in titer in the aborting ewe or a positive immunofluorescence test or darkfield microscopic examination of internal organs of the fetus or the placenta. Dihydrostreptomycin (in a dose of 25 mg/kg of body weight) has been reported to stop abortions. Vaccination with a commercial multivalent vaccine may be effective in preventing further abortions. Leptospirosis is a zoonotic disease.

Listeriosis

Listeria monocytogenes causes abortion, encephalitis, and septicemia in sheep, goats, and cattle and in humans, as well as in many other mammals.[93] *Listeria ivanovii* has been implicated only in sheep abortions.[94] *Listeria* organisms grow well in poor-quality, alkaline silage (pH > 6.0).[95] Contamination of the silage occurs from mouse, bird, or other animal feces that are inadvertently ensiled. Outbreaks also have occurred on pasture, where it is likely that the environment was were contaminated with feces containing the organism. *Listeria* causing encephalitis probably enters through the oral cavity and, after an incubation period of 2 to 6 weeks (average 30 days), travels up the facial nerves to invade the brain. Abortion occurs 7 to 30 days after oral inoculation.

Silage feeding most frequently is part of the history. Shed feeding, particularly in wet conditions, also favors infection.[94] Sheep that abort may be very ill, and metritis may follow abortion. The fetuses often are found to be very autolyzed. The organism is a facultative microaerophilic organism and difficult to grow. Tissue examination of the liver and brains of aborted fetuses, which reveals microabscessation of those organs, will help to confirm the diagnosis.

Treatment of clinical cases often is unrewarding, particularly in sheep with the encephalitic form. In an outbreak, prophylactic treatment of susceptible sheep with long-acting oxytetracycline (20 mg/kg) may avoid further cases. A live attenuated vaccine has been used in Norway to control ovine listeriosis.[96] Prevention should focus on providing only good-quality silage or avoiding silage feeding altogether. Silage should be preserved anaerobically. If bags are used, they should be kept tightly sealed. Addition of acidifying agents at ensiling time may help reduce the pH sufficiently to inhibit growth of *Listeria.* Holes may be made in bagged silage by cats' claws or by core sampling for forage analysis. Silage that appears spoiled or moldy should not be fed to sheep or goats.

Listeriosis is an important zoonotic disease. Immunocompromised people seem to be the most susceptible to infection. Most human outbreaks have occurred as a result of consuming contaminated dairy products or contaminated vegetables that were stored under cold conditions for long periods before being consumed. Contamination of dairy products usually occurs during or after processing.

Neospora canis *Infection*

Neospora canis, a protozoal parasite of livestock and companion animals, is one of the most common causes of abortion diagnosed in dairy and beef cattle. Surveys for evidence of infection with *Neospora* in aborted ovine fetuses have not shown this infection to be common in sheep.[97] As well, there are few reports in the literature of naturally occurring *Neospora* infection in sheep and none linking infection specifically to abortion,[98,99] although infection may be more common in goats.[100] Experimental infection of pregnant ewes, however, shows that this species is very susceptible to the organism, because they readily abort or deliver weak lambs and may continue to abort or deliver weak, infected lambs in subsequent pregnancies.[101,102] Histopathologic lesions are very similar to those found with toxoplasmosis abortion. Protozoal cysts can be detected in the brain of aborted fetuses using immunohistochemical staining that detects the bradyzoites. Aborted fetuses also may have a nonsuppurative myositis. Weak, live lambs also can have evidence of cerebral necrosis and meningitis. Nonsuppurative, necrotizing placentitis commonly is seen. An ELISA to detect *Neospora*-specific antibodies in ovine serum has been developed for which cross-reactivity with other protozoal infections, including toxoplasmosis, has not been observed.[103] In view of the susceptibility of sheep to this organism, *N. canis* infection should be considered as a diagnosis if toxoplasmosis is ruled out in cases of protozoal abortion. Methods of diagnosis include immunofluorescence antibody testing or ELISA of blood and PCR assay of fetal tissues.[72] If diagnosed with neosporosis, the aborting ewe should be culled.

Salmonellosis

Many species of *Salmonella* have been implicated in ovine abortion. Three species—*S. abortusovis, S. montevideo,* and *S. arizonae*—have a predilection for the ovine and often cause abortion outbreaks without significant adult disease.[104,105] Abortion rates in these outbreaks can range from very low (<1%) to as high as one third of the pregnant flock. An efficacious live attenuated vaccine against *S. abortusovis* is available in Europe for use in high-risk flocks.[106] *S. abortusovis* and *S. montevideo* are more common in Europe, and *S. arizonae* is more common in North America.

Many other species of *Salmonella* have been implicated in ovine abortion with significant adult mortality. *S.*

dublin, S. typhimurium, S. schwarzengrund, and other species have been reported to be associated with abortion and ewe death due to metritis and septicemia. One outbreak was associated with feeding lincomycin to ewes to control campylobacteriosis.[29] These organisms may be introduced through contaminated feed or by carrion birds and carrier animals such as dogs, cats, rats, and livestock. Usually these sporadic, opportunistic outbreaks disappear from the flock and do not persist to the following season.

Sarcocystosis
Abortion due to *Sarcocystis* spp. is unusual, but definitive diagnosis may be difficult. Sheep may be infected by four species of *Sarcocystis*. Two species—*Sarcocystis tenella* and *Sarcocystis arieticanis*—are pathogenic and may cause abortion during the acute-phase infection.[107] *Sarcocystis gigantea* and *Sarcocystis medusiformis* are not considered to be pathogenic to sheep. Detection of infection with pathogenic species can be done using a species-specific PCR assay on blood samples of infected sheep[108]; such testing also will help differentiate this entity from infection with other protozoal parasites of sheep. Suggested control measures include prevention of contamination of feed and pastures by feces from dogs, foxes, or other canids or cats or by preventing access of young susceptible animals to places where contamination cannot be prevented.[72]

Yersinia enterocolitica
Occasionally *Yersinia enterocolitica* (O serotype) has been isolated from ovine abortion samples—both field cases as well as experimental infections.[109] Length of incubation is 50 days. Fetuses generally are aborted dead. The placentitis is suppurative and necrotic, with thickened cotyledons. The fetuses show no external gross changes. Internal changes are consistent with microbial invasion. Thoracic and peritoneal cavities are filled with turbid serosanguineous fluid, and internal organs are hemorrhagic. The organism can be recovered from vaginal discharges up to 2 weeks after abortion. Ewes aborting from this infection can subsequently conceive and carry full-term healthy pregnancies.

NONINFECTIOUS CAUSES OF ABORTION

Stress and Trauma
Abortion can be seen after an unusual stress, such as from predator attack, shearing, or inappropriate handling. Although it is possible to handle sheep before lambing, care should be taken. Associated with inappropriate handling is the risk of fetal trauma (ruptured liver or kidney). Traumatic injury may be incurred while the ewe is in a chute or being run through a gate, but fighting and shoving between ewes at a feeder also constitute a possible cause.

Hyperthermia
Sheep that do not thermoregulate well tend to have poorer placental growth than sheep that do, although whether hyperthermia is severely detrimental to lamb survival is not clear.[110]

Energy/Protein Deficiency
At the time of breeding, energy deficiency and, to some extent, protein deficiency are associated with embryo loss. If the ewe is not fed adequately for the first and second trimesters, placental growth and attachment to caruncles will be adversely affected. Birth of small weak lambs and mummification are potential outcomes. Some abortions also may be due to in utero starvation of the fetus. Inadequate energy in the third trimester will inhibit fetal growth. Lambs will be born small, with poor body fat reserves and increased risk of hypothermia.

Overnourishing of Adolescent Ewes
If peripubertal ewes lambs are fed a balanced ration intended to promote rapid growth, a marked detrimental effect is observed on placental mass, the number of cotyledons per placenta, and subsequent birth weight of the lambs.[111] These smaller lambs experience much higher mortality rates than those for normal lambs. Laboratory data on these lambs reveal elevated serum urea, and abnormalities of the kidneys and gut are seen at necropsy. These findings underlie the need not to overfeed pregnant ewe lambs.

Astragalus *spp. and* Oxytropis *spp.*
(Locoweed) Abortion
Ingestion of locoweed can cause abortion of dead fetuses, birth defects such as arthrogryposis, right heart hypertrophy resulting in hydrops amnios and hydrops allantois, reduced birth weight, and weakness of newborn lambs.[112] The severity of the effect is dose dependent on the amount of plant ingested and the time of gestation. The toxin is the indolizidine alkaloid swainsonine. The probable pathogenic mechanisms are delay of embryo implantation, interruption of vascular development, and induction of alterations in fetal fluid balance. The toxin appears to increase prostaglandin levels in the cotyledons ($PGF_{1\alpha}$ and $PGF_{2\alpha}$) and thus decreasing serum progesterone.[113] Ingestion of locoweed also causes reduced fertility in both rams and ewes and disruption of the maternal-offspring bond.[112] It has been suggested that in some geographic regions where these plants grow commonly, namely, the western United States, locoweed ingestion is a very important cause of abortion and reproductive failure.

Veratrum californicum *Abortion*
If *Veratrum californicum* (false hellebore, California corn lily) is ingested during the breeding season, such as on day 14 of gestation, the toxin causes cyclops and arthrogryposis in the lambs.

Iodine Deficiency
If noniodized salt is fed to ewes during gestation or if cobalt-iodized salt is not made available on a free-choice basis, abortion and stillbirth due to iodine deficiency goiter may occur. The fetuses exhibit swellings on either side of the neck region that are shown to be the greatly enlarged thyroid glands. Absence of wool also is commonly seen with late-term abortions. Lambs born alive are very weak and, unless supplemented with iodine, usually die. Once diagnosed, the rest of the flock should be supplemented with oral iodine. It has been suggested

that Lugol's iodine be added to the water or that 1 to 2 ml of tincture of iodine be painted on the woolless skin once per week. To prevent problems, iodine should be fed at a rate of 0.10 to 0.80 mg/kg of dry matter (DM) diet, with the higher end of the dose range recommended for pregnant ewes and growing lambs (1985 National Research Council [NRC] recommendations). Sheep grazing goitrogenic brassicas such as forage rape or turnip tops may require higher levels of supplementation, up to 2 mg/kg of DM.

Selenium Deficiency

Selenium deficiency has been associated with early embryonic death, abortion due to in utero white muscle disease, prolonged parturition (which increases the risk of stillbirths), and white muscle disease in lambs.[114] Selenium is the only mineral that is directly associated with embryo survival. Supplementation of other minerals and vitamins may be associated with increased conception levels, but this effect probably is due to increased ovulation rate. Selenium should be added to the ration at a rate of 0.1 to 0.3 mg/kg of DM diet, with 0.25 mg/kg the probable optimal level for supplementation of sheep fed deficient rations. A sustained-release bolus containing 50 mg of selenium was associated with improved reproductive performance through an increase in both the proportion of ewes lambing and the proportion giving birth to twins.[115] The selenium also may be fed as a supplement or salt-mineral mixture to be consumed at a rate of 0.23 to 0.46 mg/sheep/day. Higher levels have been associated with toxicity unless used in a very selenium-deficient region. With oral supplementation of selenium, it takes approximately 1 month for the blood selenium levels to increase to normal levels. Parenteral injection of the pregnant ewe with commercial selenium preparations at a rate of 0.056 mg/kg of body weight at 1 month before lambing has been shown to decrease the risk of congenital and newborn nutritional myopathy.

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CHAPTER 91

Lambing Management and Neonatal Care

PAULA I. MENZIES

PRELAMBING MANAGEMENT OF PREGNANT EWES

Midgestation

Approximately 40 days after the ram is removed from the flock, scanning for pregnancy using real-time ultrasound examination is recommended to identify those animals that are not pregnant. To improve nutritional management of late-gestation ewes, fetal number also should be determined and ewes classified by body condition score.

Pregnant ewes can be grouped for feeding as follows:

- Ewes with singles in good condition
- Ewes with twins in good condition and ewes with singles in poor condition
- Ewes with triplets or more and ewes with twins in poor condition

One Month before First Expected Lambing Date

The following management procedures should be performed routinely approximately 1 month before lambing:

- Ewes should receive "booster" immunization with a multiway clostridial vaccine that includes antigens effective against tetanus, pulpy kidney (*Clostridium perfringens* type D), enterotoxemia (*C. perfringens* type C), malignant edema (*Clostridium chauvoei* and *Clostridium septicum*), and Black's disease *(Clostridium novyi)*. If the ewes have not

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- Ewes with singles in good condition
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One Month before First Expected Lambing Date

The following management procedures should be performed routinely approximately 1 month before lambing:

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had a primary series as lambs, or if the previous vaccination was administered more than 12 months previously, then a primary series should be given starting at 2 months before the first expected lambing date. A vaccine that contains antigens to *Corynebacterium pseudotuberculosis* (the agent of caseous lymphadenitis) can be included if the disease is present in the flock.

- Shear or, in sheep maintained outdoors in inclement weather, crutch (remove wool from the perineal or escutcheon area) all pregnant ewes. Shearing increases dry matter intake, is associated with improved fetal lamb growth, and improves udder cleanliness for nursing lambs.
- Increase energy and energy density of ration.
- Increase energy to ewes in poor body condition.
- Loose, clean free-choice mineral formulated for sheep, with added salt to increase palatability, should be available at all times. Special attention should be paid to providing adequate calcium if cereal hays are fed; supplemental selenium, cobalt, copper, iodine, and molybdenum if regional soils and forages are known to be deficient; and vitamin E and vitamin A if stored feeds are fed.
- If sheep are bunk fed, feeder space should allow all ewes to eat at once (i.e., a minimum of 40 cm [16 inches] of feeder space per pregnant ewe) (Table 91-1).
- If sheep are housed in a barn or dry lot, additional space must be allowed for pregnancy (a minimum of 1.4 m^2 [16 square feet] per ewe) (see Table 91-1).
- Treatment with an anthelmintic is given to prevent preparturient rise in fecal egg count. This recommendation may be modified according to regional conditions.
- If sheep are shorn or if warranted, treatment for external parasites (lice, keds, and mange) is given.
- For ewes lambing at pasture, supplementation with a coccidiostat in the mineral premix may be helpful to reduce contamination of lambing grounds.

Two Weeks before First Expected Lambing Date

The lambing area is prepared as follows:

Lambing on pasture: Straw bales are positioned to create a windbreak and to bed lambing grounds. After lambing is finished, straw can be gathered in and burned or composted. Protection against predators (e.g., guard animals) should be ensured.

Lambing inside: Ventilation must be adequate for animal numbers. Temperature fluctuations where young lambs are to be housed should be avoided. Building or pens should be cleaned out and disinfected and fresh bedding applied to lambing area.

Lambing and lamb processing equipment and medications should be available (Box 91-1).

Table **91-1**

Housing Requirements for Ewes and Nursing Lambs

Production Period	Animal Class	Housing Type	Space	Numbers
Late gestation and lambing	Ewes	Pen feeder	16 ft^2/ewe 20 inches of feeder space/ewe for limit-fed animals, 6 inches for free-choice–fed animals	<40 ewes/pen
		Waterer	25–50 ewes/bowl, 15–25 ewes/foot of tank	
1–3 days of age	Ewes and lambs	Claiming pen	4–5 ft/ewe-lamb pair	12 pens/100 ewes: natural breeding 40 pens/100 ewes: synchronized breeding
Lactation	Ewes with lambs	Pen	20 ft^2 per ewe-lamb pair; extra 5 ft^2 for prolific breeds	*2–4 days old:* 5–10 ewes-lambs/pen *5–14 days old:* 20–40 ewes-lambs/pen *14 days to weaning:* 50–100 ewes-lambs/pen
		Feeder and waterer as for gestating ewes		
Nursing and early weaning (<28 days)	Lambs	Creep area	2 ft^2/lamb 2-inches of feeder space/lamb	As for nursing lambs; 25/pen for early weaned lambs

Data from Canadian Farm Buildings Handbook, Agriculture and Agrifood Canada.

Box 91-1

Lambing Equipment and Supplies Recommended for Sheep Producers

Lambing Equipment
Clippers or hand shears for crutching ewes
Prolapse retainers and soft rope
Chlorhexidine scrub for cleaning perineum
Clean terrycloth towels
Two clean buckets: one for warm water to wash, one for cold water to revive lambs
Sterile lubricant (e.g., K-Y jelly)
Plastic disposable rectal sleeves
Nylon lambing ropes and lamb snare

Identification Equipment
Paint for spraying/branding lambs
Ear tags and tagger
Tattoo and ink (optional)
Lambing diary

Lamb Feeding Equipment
Tube feeding kit with rubber tube and 60-ml syringe or 250-ml squeeze bottle
Source of frozen colostrum (bovine, caprine, or ovine)
Lamb milk replacer with nipples and bottles
Lamb bar (optional)
Equipment for fostering lambs (e.g., head gate)

Lamb Processing Equipment
2.5% iodine for painting umbilical cord stump
Sterile syringes: 1-ml, 3-ml, and 60-ml
Sterile needles: 22-gauge and 20-gauge, 1-inch
Empty Javex bottle with lid for sharps disposal
Warming box for chilled lambs
Digital thermometer with a readout to 36° C
Equipment for tail docking and castration (rings, hot-docker, Burdizzo emasculators)

Medications*
Propylene glycol and drench gun or drench bottle
Injectable vitamin E and selenium product labeled for newborn lambs
Multivalent clostridial vaccine
Tetanus antitoxin (optional)
Contagious ecthyma vaccine (optional)
Calcium borogluconate for subcutaneous administration
50% dextrose to be diluted for treatment of hypoglycemic lambs
Oral electrolyte (registered for calf use)
Antibiotics: penicillin and long-acting oxytetracycline
Larvicidal anthelmintic

*Provided for clients with an established relationship with the practitioner.

PARTURITION (LAMBING)

Gestation length varies considerably with breed of sheep. It also varies within breed with number of fetuses being carried. Triplet-bearing ewes usually lamb before single-bearing ewes bred on the same day. On average, sheep gestate for 145 days. Normal lambings have been reported as early as day 137, but generally sheep do not lamb earlier than day 142.

At 1 week before the first expected lambing date (day 138 after the ram is introduced to the breeding flock), ewes should be observed every 4 to 6 hours. Diseases to observe for include signs of pregnancy toxemia, which include refusal to eat grain, opisthotonos, teeth grinding, fine muscle tremors, and coma; signs of hypocalcemia, which include stilted gait, excessive salivation, and recumbency with hind legs extended behind; vaginal prolapse; abortion; mastitis; and rupture of the prepubic tendon. If any of these conditions occurs, prompt treatment should be instituted.

Signs of the **first stage of labor** include udder enlargement and engorgement with colostrum, relaxation of the pelvic ligaments, and vulvar swelling. The ewe will separate from the flock and begin nesting. If on pasture, she will seek an isolated spot with slight elevation or close to a fence line. Ewes may seek shelter, if it is provided, in order to protect their newborn lambs from inclement weather. Within a paddock, ewes may choose the same location in which to give birth, presumably attracted by the scent of birth fluids. In a pen, ewes may seek a corner or an open claiming pen. She will circle, paw at the straw, and bleat in a low voice. She will stop to smell any uterine or vaginal discharges. As the uterine contractions become stronger and the fetus moves up into the pelvis, she may lie down repeatedly and strain with the head raised.

The **second stage of labor** generally takes less than an hour from the start of intense straining to the delivery of the lamb. Time to delivery of lambs is approximately 30 minutes or less for a single lamb and up to 2 hours for triplets. Labor in primiparous ewes may take longer. No preference in time of day to deliver has been observed, but if disturbed, the ewe may stop labor for a few minutes until settled again. Normal presentation of the lamb is described as anterior dorsosacral with front limbs extended and preceding the nose by approximately 6 cm (2 to 4 inches). Also normal but associated with greater risk to the safe delivery of the lamb is posterior dorsosacral with hind limbs extended. If delivery is delayed and the umbilical cord is compressed between the lamb and the pelvis of the ewe, the lamb may suffocate before delivery is complete. After delivery of the lamb, the ewe stands, causing the umbilicus to rupture. The ewe turns and begins to nuzzle and lick the lamb to stimulate breathing and to clean it off.

INDUCTION OF PARTURITION

Induction of lambing can be done if the breeding date is known within an accuracy of 3 days—for example, if breeding was synchronized using hormones and only one breeding opportunity occurred. Ewes will respond to an injection of dexamethasone (16 mg given intramuscularly [IM]) or betamethasone (10 to 12 mg IM) after day 137, but it is preferred to wait until day 142 to ensure good fetal viability. Commonly, producers may wait for the first few lambs to be born and then induce the rest of the pregnant ewes. Lambing generally occurs between 36 and 60 hours after induction. Induction is not associated with an

increased risk of retained fetal membranes. In instances of vaginal prolapse or pregnancy toxemia, it may be advisable to induce as early as day 137, with the goal of saving the ewe.

MANAGEMENT OF DYSTOCIA

Common Causes of Dystocia

Causes of dystocia may include the following:

- Malpresentation of the lamb
- Maternofetal disproportion
- Poor cervical dilation
- Uterine torsion
- Uterine inertia

Malpresentation of the lamb (or lambs) may manifest in any of various ways: one or both front limbs retained; head retroflexed; true breech presentation, in which the lamb is in the posterior dorsosacral position but the hind legs are flexed forward; dorsopubic or dorsoiliac position; and in the case of multiple births, presence of multiple limbs or heads, or of more than one lamb, in the pelvis at the same time.

Maternofetal disproportion most commonly occurs when ewes that are carrying singles are fed too well in late gestation. Occasionally the ewe may simply have a congenitally small pelvis, or the disproportion may be the result of mating a small-breed ewe and a large-breed ram.

Poor cervical dilation may be due to vaginal prolapse; improper presentation, position, or posture of the lambs; fetal death before stage 1 labor (e.g., from abortion diseases); or possibly damage to the cervix from chronic exposure to phytoestrogens. **Ringwomb** is the term used to describe incomplete cervical dilation that is unresponsive to manual dilation. The cervix is hard, as it is before stage 1 labor. This condition occurs more frequently in primigravida lambs but may be seen in sheep of any age. Most flocks experience occasional cases of ringwomb, but outbreaks in which up to 15% of ewes are affected have been described.

Uterine torsion occurs occasionally and can be diagnosed on vaginal examination. It may be confused with poor cervical dilation.

Primary **uterine inertia** may occur if the ewe is ill (e.g., from hypocalcemia or pregnancy toxemia); secondary inertia occurs when the ewe is exhausted after a prolonged labor.

Correction of Dystocia

The practitioner should ensure that the proper tools and protection are available and that the gloves are well lubricated (see Box 91-1). The uterine wall is friable, so only gentle manipulation is advised. A tear in the uterus leads to peritonitis, which is not well tolerated in sheep.

If extensive manipulation is required, use of an epidural anesthetic procedure is advised. The epidural injection generally is performed at the sacrococcygeal space. After the area is clipped and disinfected, a 20-gauge needle is inserted at an angle of 20 degrees to the tail when held horizontally. Lidocaine hydrochloride is then injected at a dose rate of 0.5 mg/kg of body weight (1 ml of 2% lidocaine/40 kg). Additional analgesia may be obtained by combining xylazine with the lidocaine hydrochloride at a dose of 0.07 mg/kg of body weight (0.14 ml of 2% xylazine added to each milliliter of lidocaine hydrochloride). Adult ewes may range in mature body weight from 60 to 80 kg, depending on breed and maturity. This dosage may cause paresis and ataxia of the hindlimbs for up to 8 hours.

With a *retained front limb,* if the lamb is small, sometimes it may be extracted by gently pulling with the leg still back. With large lambs, however (or small ewes), it is necessary to correct the limb position before delivery is attempted. It generally is necessary to repulse the lamb gently back into the uterus if possible and then, after tracing the limb from the shoulder and elbow, hook the retained leg below the elbow with a finger, bending the carpus and then the fetlock in order to raise the foot over the brim of the pelvis. The practitioner must take care to protect the wall of the uterus from the foot by cupping the foot in the hand. If the fetus cannot be repulsed or if room in the pelvis is insufficient to allow correction of the position, a cesarean section is indicated if the lamb is still alive, or if the lamb is dead, a partial fetotomy is indicated. Viability is determined by checking for the following: suckle reflex, gag reflex, corneal reflex. If none are present, a partial fetotomy is performed by removing the head and neck to increase room to reposition the leg.

With *lateral retroflexion of the head,* again, an attempt is made to repulse the lamb in order to increase the available space for correction of the head position. A lamb snare around the head and front limbs can be used to help guide the head around. Traction on the lower jaw is avoided unless the lamb is dead, because this usually will result in a fracture to the mandible. If this maneuver is unsuccessful, cesarean section if the lamb is alive or partial fetotomy if the lamb is dead should be considered.

With *multiple fetuses,* care should be taken to trace limbs and head back to the body and identify which limbs belong to which lambs before repulsion or extraction is attempted.

Maternofetal disproportion may be present concurrently with malposture (e.g., head or limb retroflexion). Once the lamb is in the proper presentation, position, and posture, either soft ropes or the lamb puller can be used to apply gentle traction, ideally to both the front limbs and the head simultaneously. To determine if the lamb can be delivered vaginally, the examiner can assess whether both elbows and the head can be pulled into the pelvis at the same time. Inability to do so with relative ease indicates that vaginal delivery cannot be done safely for the ewe or the fetus. Plenty of lubrication is used to assist extraction. While the lamb is being pulled, after the thorax is clear of the vagina, the lamb is rotated approximately 30 degrees to prevent hip lock.

With *poor cervical dilation,* the practitioner attempts gentle manual dilation, keeping the hand and arm well lubricated. If after 10 minutes no progress is observed, the problem may be true ringwomb. In this condition, the cervix does not undergo the normal parturient softening.

The cervical softening process starts with the prepartal drop in progesterone. This triggers an infiltration of leukocytes, which causes collagen degradation and hence softening. The cause for failure of the cervical softening process is not known. Some success at treatment has been reported with application of an estrogen product or prostaglandin $E_{2\alpha}$ to the cervical area, or with injection of such agents, and followed in a few hours by oxytocin. The alternative is cesarean section.

With *uterine torsion,* the practitioner must determine the direction in which the torsion has occurred and then either gently flip the uterus around or hold the fetus and have assistants roll the ewe in the opposite direction of the torsion. In approximately 50% of the cases, poor cervical dilation is present even after detorsion has been accomplished. Cesarean section should be considered in those cases.

If *uterine inertia* is due to hypocalcemia, the ewe is given parenteral calcium before delivery. This is done by slow intravenous administration of 50 to 100 ml of a commercial calcium borogluconate solution, followed by an additional 50 to 100 ml injected subcutaneously.

After delivery of the lamb primarily responsible for the dystocia, oxytocin (30 to 50 IU) can be given (unless the dystocia is due to uterine inertia). After 10 minutes, the vagina is checked for the presence of additional lambs. If uterine inertia is present, both horns are carefully explored to their ends to locate more lambs.

Cesarean Section

Anesthesia for performing a cesarean section can be obtained using one of the following methods.

A line or inverted "L" block of 2% lidocaine (without epinephrine) can be given on the left flank if a flank laparotomy will be performed, or given ventral midline if that approach will be used.

For the left flank only, paravertebral anesthesia to block the T13, L1, and L2 paravertebral nerves is achieved using the following method: The midpoint of the first lumbar process is confirmed by palpation, and a 6-cm spinal needle is inserted 2.5 to 3.0 cm from the midline along a perpendicular line running from the midpoint of the first lumbar transverse process to the spine and at a depth of 4.0 to 5.0 cm. The needle may need to be directed forward to walk it off the front of the transverse process. After the needle is felt to penetrate the ligament between T13 and L1, 4 to 5 ml of 1% lidocaine hydrochloride is injected to block the 13th thoracic nerve. the needle is retracted and an additional 2 ml is injected to block the dorsal branch of the T13 nerve. The needle is then withdrawn and redirected caudally to block L1 and is walked off the back of the first transverse process. Another 4 to 5 mL is given; then the needle is withdrawn slightly and another 2 ml is injected. To block L2, this technique is repeated but just anterior to the second lumbar process.

An epidural using lidocaine, xylazine, or a combination of both (as described previously) may be used in conjunction with a line or inverted AL block.

Surgical technique is standard. If the fetuses are dead, the uterus should be packed off using sterile drapes before an incision is made. After the first lamb is extracted, both horns should be carefully explored to the tip to detect the presence of more lambs. The practitioner should avoid incising any caruncles because this may result in excessive bleeding. After suturing, oxytocin is administered once and parenteral antibiotics are given for 3 to 5 days.

Fetotomy

Not uncommonly, a dystocia may not be detected until the lambs have died, or in some instances, have become emphysematous. If the lambs cannot be pulled, a fetotomy is preferred to a cesarean section. If the fetus is emphysematous, the uterus also may be compromised and friable. A subcutaneous fetotomy can be performed using a finger knife to avoid vaginal or uterine damage. Again, oxytocin and antibiotics are given at the completion of the procedure.

CARE AND MANAGEMENT OF THE NEONATAL LAMB

Resuscitation of the Newborn Lamb

Lambs normally will begin to breathe within 30 seconds of delivery. Techniques to stimulate the lamb that is slow to breathe include rubbing the head and thorax vigorously with a dry towel, pouring cold water in the ear, and stimulating the sneeze reflex by tickling the nostrils. Swinging the lamb may help extract fluid from the nose but makes it difficult for the lamb to breathe because of centrifugal pressure on the diaphragm. Mouth-to-nose resuscitation should not be done because of the risk of contracting a zoonotic infection from the lamb, such as *Chlamydophila abortus* or *Coxiella burnetii* infection. A technique of inflating the lungs that has been used with some success is as follows: A lamb stomach tube is inserted into the esophagus of the lamb. By means of gentle pressure applied with a thumb and forefinger, the esophagus is closed off distal to the end of the tube. The other hand is used to close off the mouth and nostrils. The clinician softly blows once into the tube. With the esophagus closed off, the air will be forced down the trachea into the lungs. The tube is removed after delivery of one breath, and the lamb is reassessed.

Normal Behavior at Birth

Lambs should be standing within 10 to 20 minutes of birth. The normal cleaning behavior of the ewe stimulates them to stand. They are then attracted to the ventral line of the ewe's abdomen and will follow it down to where it meets the udder. The ewe's licking and nudging behavior encourages a suckle reflex. A low udder or an udder covered with wool, or pendulous, misshapen teats, will interfere with successful nursing. The licking also serves to provide olfactory stimulation to aid in maternal recognition of the lamb. This bonding generally occurs in the first hour after birth. It is critical that the ewe have an undisturbed opportunity to bond with her lambs, without the risk of attempts at stealing them by another

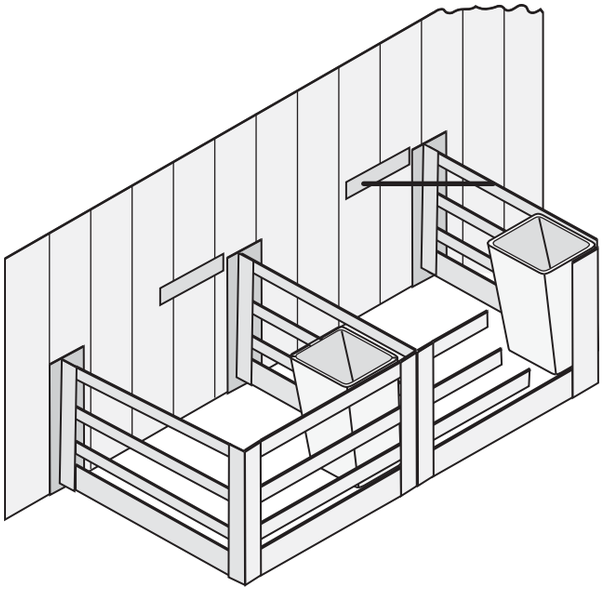

Fig. 91-1 Plan for a claiming pen. (Courtesy of Ontario Ministry of Agriculture and Food, Ontario, Canada.)

ewe. Circling behavior is abnormal and indicates that the bonding process is failing. Failure to bond and failure to successfully find the teat are major risk factors for hypothermia/hypoglycemia and septicemia.

The chance of successful bonding will improve if the ewe and lambs are provided with a quiet area, undisturbed by other sheep. Mothering is optimized by confining them in a claiming pen (jug) for 12 to 24 hours. The producer can more easily check the status of the lambs (stomach fill and attitude) and the behavior of the ewe (for signs of rejection) at the time when lambs are most at risk. Claiming pen size should be a minimum of 4 feet by 4 feet; for larger breeds or ewes with multiple lambs, 4 feet by 5 feet is preferred (Fig. 91-1). The bottom panels should be spaced close together to prevent the lamb from escaping. Ewes should be provided with fresh water and feed. Heat lamps are optional and should not be used once the lambs are dry.

Passive Immunity

If the ewe is fed correctly in late gestation, is properly vaccinated, and does not have mastitis, she should be able to provide the lamb with colostrum of adequate quantity and quality. Exceptions to this rule of thumb may be observed with maiden ewes and with ewes that have more than two lambs. If the ewe has inadequate colostrum or if the lamb is too weak to nurse properly, then the lamb should be fed colostrum at a rate of 50 ml/kg of body weight within 2 hours of birth and at rate of 200 to 250 ml/kg of body weight over the first 24 hours of life. If the lamb is weak, the colostrum should be administered by stomach tube.

Alternate-Source Colostrum

If the ewe has inadequate colostrum or is considered a risk to pass on specific diseases (e.g., ovine progressive pneumonia [maedi-visna], Johne's disease), colostrum obtained from other animals can be fed. Most ideal is sheep colostrum from a ewe of known health status. Colostrum may be frozen up to 6 months without loss of quality. It should be frozen in 50-ml (approximately 2-ounce) aliquots to facilitate thawing. Rapid thawing with heat (e.g., microwave on high) will denature the immunoglobulins. It is recommended initially to freeze the colostrum in an ice cube tray and then, once it is frozen, to transfer the cubes to a freezer bag labeled with the donor's identification data and the date of freezing. Only first-milk colostrum should be used.

Bovine and caprine colostrum also may be used, but associated risks have been recognized. Ideally, source animals should not be infected with infectious agents that may cause disease in sheep, such as bovine leukosis virus, *Mycobacterium avium* spp. *paratuberculosis,* bovine viral diarrhea virus, caprine arthritis encephalitis virus, and *Mycoplasma mycoides* spp. *mycoides*. The quality of the colostrum can be improved by vaccinating the donor animals against clostridial diseases.

Anemia due to an unknown factor in cow colostrum has been reported in lambs 1 to 3 weeks of age. This factor appears to increase the rate of red blood cell destruction both in circulation and in the bone marrow of affected lambs. They appear weak and pale. Although many recover without treatment, those severely affected, as indicated by a packed cell volume less than 10%, can be transfused by administering blood (10 ml of whole blood with anticoagulant per kg of body weight given intravenously or by intraperitoneal instillation).

Antibodies obtained from nonhost species may have a shorter half-life. A consequence of this limitation may be a higher risk of infectious diseases such as pneumonia. This also is true of commercial colostrum supplements, which are made from bovine sources. Whey-based products may not contain adequate levels of immunoglobulins. It is not known if serum-derived colostrum supplements provide sufficient protection to lambs.

Umbilical Care

Many opportunistic pathogens may gain entry through the stumps of the umbilical veins or arteries. A solution of 2.5% iodine in an alcohol base should be used to dip the entire navel soon after birth. Solutions containing a higher concentration of iodine may cause chemical burning and inflammation. Products containing glycerin (e.g., commercial teat dips) should not be used, because these prevent drying of the umbilical tissue. Paper disposable cups should be used, and excess dip and the cup discarded afterward.

Vitamin E and Selenium Supplementation

If the flock is located in a selenium-deficient area and the pregnant ewes have not been adequately supplemented, newborn lambs should be injected with a commercial vitamin E plus selenium preparation. The single dose of

0.75 mg of selenium, given by the intramuscular or subcutaneous route, should not be repeated. Toxicity and death have been associated with as little as 1.0 mg of selenium. It is safer and more effective to supplement pregnant ewes at a rate of 0.1 to 0.3 mg of selenium/kg of dry matter (DM) total ration, depending on the natural levels in the feed and governmental restrictions. If the ewe is to be injected with a commercial product rather than supplemented in the feed, 3 mg/45 kg of body weight is given up to every 2 weeks in the last trimester. Only products labeled for use in adult sheep should be used. Although vitamin E does not cross the placental barrier, it should be supplemented to the pregnant ewe at 15 IU/kg DM total ration (1.5 IU = 1.0 mg/dl of α-tocopherol). This will increase vitamin E levels in the colostrum. Lamb rations should contain 20 to 40 IU vitamin E/kg DM total ration. Vitamin A and D injections can be administered to gestating ewes if the ration is deficient.

Identification

Each lamb should be assigned a specific identifier at birth for two reasons: to identify lambs to retain as replacements at a future date and to identify ewe-lamb pairs. Methods include ear tagging, paint branding, and spraying on the back or side with the lamb's identification, the ewe's identification with or without the birth order number and litter size (e.g., 132P-13 would be the first-born lamb of ewe 132P out of a litter of triplets, and 132P-33 would be the third-born lamb of ewe 132P), or a unique design (e.g., a spiral). The ewe also should be paint-branded at the same time—for example, 132P-3 means that she has 3 lambs at foot. Permanent identification (tag or tattoo) can be given to lambs at birth or at weaning.

Contagious Ecthyma Vaccination

If the flock has significant problems caused by contagious ecthyma virus infection (mastitis, lamb losses, or diminished productivity), then it may be advisable to vaccinate the lambs at birth with a commercial vaccine (if available). The vaccine should not be used in unaffected flocks and need not be used if the disease is only mildly manifested in the flock.

Taildocking and Castration

Taildocking should be routinely performed in all long-tailed breeds within the first 7 days and preferably within the first 24 hours of life, but only after the lamb has received adequate colostrum. Use of either rubber bands or an electric docker is the preferred method. The tail should be docked distal to the end of the tailfold and should be long enough to cover at least the vulva in a ewe lamb. Short docking has been associated with increased risk of rectal prolapse and spinal abscesses.

Castration need not be performed in ram lambs to be marketed before puberty, to take advantage of the improved feed conversion rates of intact rams. If ram lambs are to be castrated, surgery should be performed before 7 days of age if the rubber rings or cut-and-pull technique will be used, and before 90 days of age if the spermatic cord will be crushed. Lambs castrated with rubber rings after 7 days of age should have the cord crushed first in order to destroy the nerves. This appears to reduce the pain associated with that procedure. Lambs born to inadequately vaccinated ewes should be given tetanus antitoxin (250–300 IU administered subcutaneously) at the time of docking or castration. Analgesia at the time of castration or taildocking has been used with variable success. Injection of long-acting local anesthetics proximal to the surgical site may offer temporary relief. Postoperative analgesia may be best provided by administering xylazine before surgery, but animals should be constantly observed until the sedative effects have worn off.

Nutrition of the Nursing Lamb

Peak milk production in ewes occurs approximately 30 days after lambing, and ewe nutrition in the early lactation period should be geared to maximize this production. Ewe's milk provides most of the nutrient requirements for lambs until they reach 30 to 45 days of age. At 10 days of age, lambs will start to consume small amounts of solid feed, but depending on the availability of ewe's milk, intakes of solid feed are low until weaning. To increase intake of solid feed, texturized 16% protein "creep feed" should be available, consisting of mixed grain, pellets containing minerals and medications, and a binding compound, commonly molasses. Feeders should be structured to prevent fecal contamination and wastage of feed. Restricted-access turkey or pig feeders work well for lambs.

Fostering or Artificial Rearing of Lambs

Indications

Indications for fostering or artificial rearing of lambs include the following:

Dam-related: Death or severe illness may require separation of the lamb from its dam, or the dam may reject the lamb. Other causes may include mastitis or other reason for insufficient milk production, poor udder conformation (too low or teats malformed), and birth of too many lambs to be reared by their dam; or another dam that has lost all her lambs is available as a foster mother.

Lamb-related: The lamb is unable to nurse its dam effectively because of weakness or congenital deformity.

Owner-specific: The producer prefers not to have dams rear more than 2 lambs, or not to have dams rear singles, to make nutritional management easier.

Artificial Rearing of the Nursing Lamb (First 4 Weeks)

Most orphan or rejected lambs can be raised on milk replacer until the age of 21 to 28 days, when they can be weaned onto a high-quality creep ration such as described earlier. Lamb milk replacer is higher in fat than calf or kid milk replacer, so the latter should not be used to rear

lambs. An example of a good milk replacer is one containing 22% protein from milk sources, 28% fat from animal sources, and 24% lactose. Milk replacer should be fed cold (4° C) and *ad libitum* from a lamb bar to prevent engorgement and abomasal bloat. In absence of a lamb bar, lambs can be bottle fed from a nipple bottle but should be fed frequently (e.g., 4 times per day). If abomasal bloat losses occur, formaldehyde added to the milk replacer (1 ml/L of milk) will decrease losses.

Foster Rearing

When cross-fostering is used, if at all possible the weakest lambs should be left with the dam, and all fostered lambs should have received adequate colostrum first. Successful fostering of lambs may be accomplished in any of several ways, but ultimately the attitude of the foster dam often determines the success. Attitude may be individual or may be breed determined.

Interventions used to promote successful fostering include the following:

- Manipulating the ewe's detection of scent by skinning the dead lamb and tying the hide to the foster lamb; washing the foster lamb in birth fluids or rubbing it with the fresh placenta; applying various scent blocks to the nares of the ewe.
- Manipulating the ewe's maternal instinct by cervical or vaginal stimulation; removing her own lamb and returning the foster lamb back with her at the same time; "hog-tying" the back leg of the foster lamb to mimic the behavior of a newborn lamb; bringing in a dog to "threaten" the ewe.
- Restraining the ewe so that the lamb can successfully suckle until the ewe accepts the lamb. A stanchion or head gate is the most commonly used device to prevent the ewe from circling. She can still kick, however, so the lamb should be watched to ensure it is able to nurse.

PREVENTION OF PERINATAL LAMB MORBIDITY AND MORTALITY

The perinatal period extends from the first stage of labor. The end of this period is less well defined but this phase can be divided into periods in which specific causes tend to exhibit themselves more commonly. For investigating lamb mortality, losses often are divided into those deaths that occur ante partum, during parturition, and at less than 5 to 24 hours (i.e., until the "mothering-up" process has finished, when bonding has occurred and lamb has ingested colostrum), 5 to 24 hours up to 48 hours (i.e., before acquired infections after birth have caused disease), and beyond 2 days. After 2 days of age, perinatal events can still influence mortality, but effects generally are not observed beyond 2 weeks of age.

Excessive lamb mortality and stillbirth are important limitations on productivity. Stillbirth losses (number of lambs born dead of all lambs born) should not exceed 5%. Lamb mortality levels of greater than 15% before weaning are not uncommon, although with good management, it is possible to keep losses below 5%. Box 91-2 lists possible causes of perinatal lamb mortality.

Box **91-2**

Common Causes of Perinatal Lamb Morbidity and Mortality

Prepartal abortion diseases responsible for weak or stillborn lambs: infections due to *Chlamydophila abortus, Campylobacter fetus* spp. fetus and *Campylobacter jejuni, Coxiella burnetii,* and *Toxoplasma gondii;* border disease; iodine deficiency
Dystocia responsible for birth trauma such as:
  Fractured ribs and long bones
  CNS lesions: subdural hematomas, particularly in the posterior brainstem; petechiation in brain associated with hypoxia and anoxia during the birth process; lesions may not manifest as neurologic disease but as depression and difficulty with thermoregulation
  Ruptured liver, spleen, kidney
  Edema of entrapped limb/head
Starvation, hypoglycemia
Chilling, hypothermia
Infectious diseases: abortion diseases as outlined above; septicemia due to failure of passive transfer; other septicemias (e.g., listeriosis); omphalophlebitis; pneumonia; diarrhea; joint ill (chlamydiosis, erysipelas)
Nutritional deficiencies: vitamin E, selenium, copper, iodine
Predation
Trauma from misadventure
Congenital malformations: atresia ani, contracted tendons, cleft palate
CNS, central nervous system.

Investigation of Perinatal Lamb Mortality

A flock health management program should include routine necropsy of all aborted fetuses and stillborn and dead lambs. A simple form can be used to assist in collecting all necessary history and data (Fig. 91-2). Findings on gross necropsy can easily be used to diagnose why the lamb died, and to define the management area that is responsible for the loss of the lamb.

History

The history should include details of the farm environment and management, as well as ewe and lamb factors. Wet and cold weather can play an important role in lamb losses. Older ewes or ewe lambs may be at greater risk for losing lambs. In excessively thin ewes, risks are greater for production of small lambs, prolongation of labor, and production of inadequate amounts of milk. Litter size, birth weight, and history of dystocia all are important risk factors in lamb mortality. Routine management procedures should be noted, as well as any treatment lambs have received. Notations of previous losses will help to identify problem areas.

Examination of the External Carcass

The examiner should make note of specific abnormalities:

- Atypical condition of coat: abnormal quality and amount of wool, indicating premature birth or congenital infection with border disease virus; meconium staining, indicating stressful birth;

ONTARIO
SHEEP HEALTH PROGRAM

First Name _____ Last Name _____

Veterinarian's Name _____

Date of Visit _____ Producer OSHP # _____

LAMB PERINATAL MORTALITY INVESTIGATION FORM

Lamb I.D.: _____ **Litter Size:** _____ **Date and Time of Birth:** _____

Lambing: Unassisted Easy Hard Not Observed **Date and Time of Death:** _____

Ewe I.D.: _____ **Ewe Age:** _____ **Body Condition Score:** 1 2 3 4 5 (Circle one)

Birth Wgt: _____ (kg/lb) **Littermate Wgts:** _____ (kg/lb)

HISTORY:

Date of Start of Lambing Season: _____

Weather Last 24 Hrs: Adverse Indifferent Fair (Circle one) **Barn Conditions:** Good Fair Bad (Circle one)

Other Comments on History (including any treatments, other losses)**:**

SUMMARY

Age of Lamb at Death: (from necropsy observations and history):

PREPARTUM	PARTURIENT	EARLY POSTPARTUM Birth to 24 hrs. (Circle one)	LATE POSTPARTUM 24 to 48 hrs.	NEONATAL 2–30 days

Diagnosis:

Recommendations:

A

Fig. 91-2 **A** and **B,** Example of a necropsy form for investigating lamb perinatal mortality.

adherent placenta, indicating lack of cleaning by the ewe
- Saliva staining around the mouth, indicating endotoxemia (watery mouth)
- Congenital abnormalities (e.g., spider lamb, atresia ani, intersex, cleft palate, arthrogryposis)
- Perineal staining from diarrhea

The footpads are examined to determine whether walking occurred before death, as indicated by some "wear" of the pads; intact footpads signify that the lamb never walked.

Skinning back the carcass, the examiner should note the following:

- Degree of dehydration, keeping in mind that length of time since death and freezing may confound accurate observation
- Subcutaneous edema (regional or generalized); ascites, anasarca, or swollen head due to dystocia

NECROPSY FINDINGS

✓ relevant findings in appropriate box

EXTERNAL APPEARANCE:

☐ Meconium stained
☐ Wool coat not cleaned
☐ Foot pads worn (walked)
☐ Congenital abnormalities
☐ Perineal staining (diarrhea)
Other observations:

SUBCUTANEOUS APPEARANCE:

☐ Edema generalized
☐ Edema localized (e.g., head)
☐ Petechial hemorrhages of limbs
☐ Dehydration
☐ Enlarged thyroid glands (goiter)
Other observations:

UMBILICUS:

☐ Pointed with clot adhered
☐ Pointed with clot
☐ Blunt–no clot
☐ Omphalophlebitis
Other observations:

FAT: (EPICARDIAL/RENAL)

☐ Present
☐ Partially metabolized
☐ Fully metabloized (absent)
Other observations:

INTESTINES:

☐ Empty
☐ Normal
☐ Milk fat in mesenteric lymphatics
☐ Diarrhea
Other observations:

LUNGS:

☐ Inflated
☐ Partially inflated
☐ Atelectic
☐ Aspiration
☐ Pneumonia
Other observations:

ABOMASUM:

☐ Empty
☐ Milk/Colostrum
☐ Solid feed
☐ Foreign debris
Other observations:

OTHER:

CNS (hemorrhages):
Pleural/Peritoneal Cavity:
Musculoskeletal:
Kidneys:
Adrenal Glands:
Other observations:

SUMMARY OF RELEVANT FINDINGS:

B

Fig. 91-2, cont'd

- Bilateral swelling on the neck, which may be an enlarged thyroid, suggesting goiter due to iodine deficiency
- Umbilicus, to determine whether the umbilicus is blunt and arteries are empty, evidence that the lamb died before or during the birth process; is tapered and contains blood, evidence that the lamb survived the birth process; or is tapered and contains an organized adherent clot, evidence that the lamb survived the birth process by several hours. Hyperemia, fibrin, and purulent material in the umbilical region indicate omphalophlebitis.
- The skeletal muscles, which should be checked for evidence of white muscle disease

The skin should be removed from the limbs and the subcutaneous tissues examined for hemorrhages, which indicate that the lamb was hypothermic before death.

For internal examination, the lamb carcass should be opened with its right side down and the organs examined in situ. The following should be noted:

- Evidence of pleural or peritoneal fibrin, indicating a septic condition before death. Swabs of this fluid should be submitted for culture. If an agent associated with abortion is suspected, abomasal contents and thoracic fluid or heart blood should be collected using sterile needles and syringes (see Chapter 90).
- Blood in the peritoneal cavity, which may be from a ruptured liver, kidney, or mesenteric or umbilical vessel and suggests trauma occurring in utero, during parturition, or post partum, depending on other findings.
- Blood-tinged fluid in the peritoneal or pleural cavity, which may indicate that the lamb died in utero 1 or 2 days before delivery.
- The amount of brown fat around the kidney and heart, which should be evaluated. Serous atrophy suggests that the lamb was hypoglycemic before death. Heart fat is depleted first, then kidney fat.
- Aeration of the lungs, which can be assessed quickly by dropping a piece of lung tissue in a jar of water to assess buoyancy, and evidence of pneumonia. Total atelectasis of the lungs indicates that the lamb never breathed. The trachea and bronchi should be opened to examine for aspiration pneumonia (the owner may have incorrectly passed a stomach tube).
- The tongue, diaphragm, and heart should be sectioned and examined for evidence of white muscle disease. The heart should be checked for congenital defects.
- The abomasum should be examined for type and quantity of content. An empty abomasum or an abomasum that contains dirt or grass, along with the finding of serous atrophy of fat, suggests that the lamb survived at least 5 hours but never nursed. Detection of milkfat in the mesenteric lymphatics suggests that several hours have passed since feeding.
- The skull and the brain, particularly the atlanto-occipital region, should be examined for hemorrhages, both subdural and throughout the brain, indicating the possibility of birth trauma.
- The joints all should be opened to look for evidence of septic arthritis.

By using the information gathered on gross necropsy (Fig. 91-3), the death of the lamb can be classified as follows (Fig. 91-4):

Prepartum—early. The lamb appears obviously premature, with decreased or absent wool coat. This should

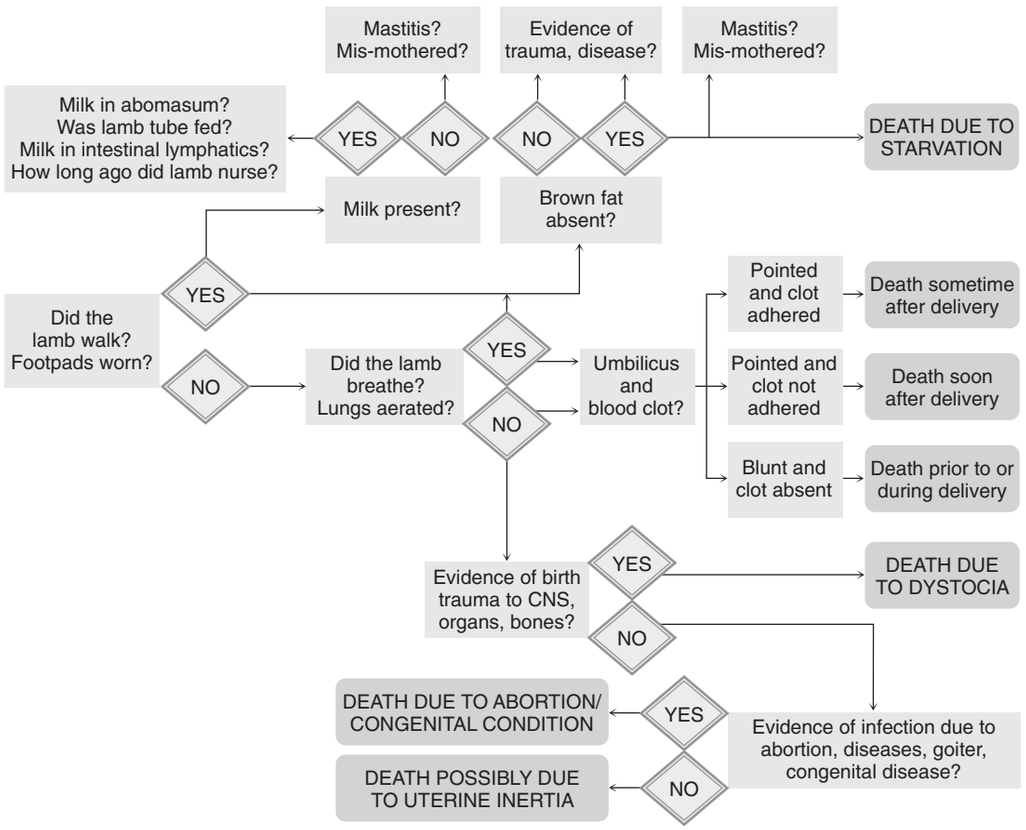

Fig. 91-3 Algorithm for classification of lamb perinatal mortality.

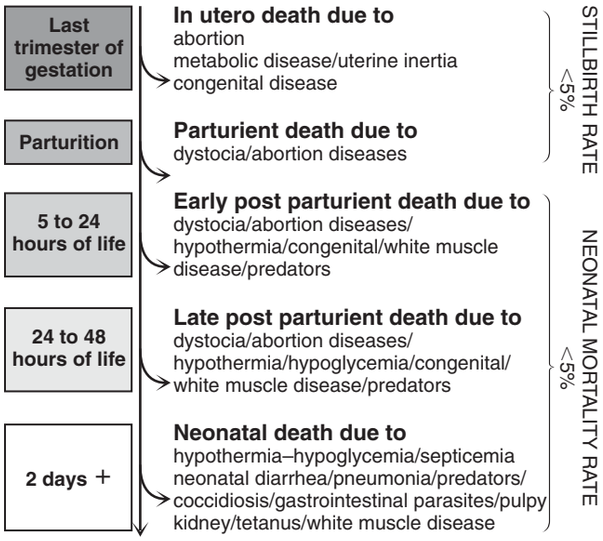

Fig. 91-4 Classification of timing of death in lambs and common causes of mortality.

be considered an abortion and investigated as such (see Chapter 90).

Prepartum—late. The lamb appears to be term but was delivered stillborn, with no evidence of birth trauma. Although the lamb's death may have been from delayed parturition due to uterine inertia , it is advisable to investigate the death as an abortion.

Parturient. Evidence of birth trauma is present, and the lungs are atelectatic.

Postparturient, early. Death occurred after birth but most often before 5 hours of age—up to 24 hours. Brown fat stores are evident, and the lungs are partially or fully inflated. The lamb may have been weak when born because of infection with abortion agents, may have succumbed to birth-related injuries, with or without evidence of hypothermia, or may have been primarily hypothermic.

Late postparturient, older than 5 to 24 hours. After 5 hours of age, lambs start to deplete their brown fat stores if they do not receive adequate nourishment (colostrum). Mismothering, inadequate colostrum supply, and weakness due to low birth weight or birth trauma will predispose the lamb to starvation (hypoglycemia). Up to 48 hours of age, death often is related to primary hypoglycemia with secondary hypothermia.

Neonatal, older than 48 hours. Although death after 48 hours may still be due to hypothermia or hypoglycemia secondary to birth trauma, mismothering, and other causes, after this time infectious diseases also can occur.

Recognition and Treatment of Weak Lambs

It is important to instruct the producer on how to recognize a sick lamb and how to apply appropriate nursing care. Hypothermia and hypoglycemia are the most common causes of lamb mortality; if recognized early, both are responsive to treatment. Affected lambs initially

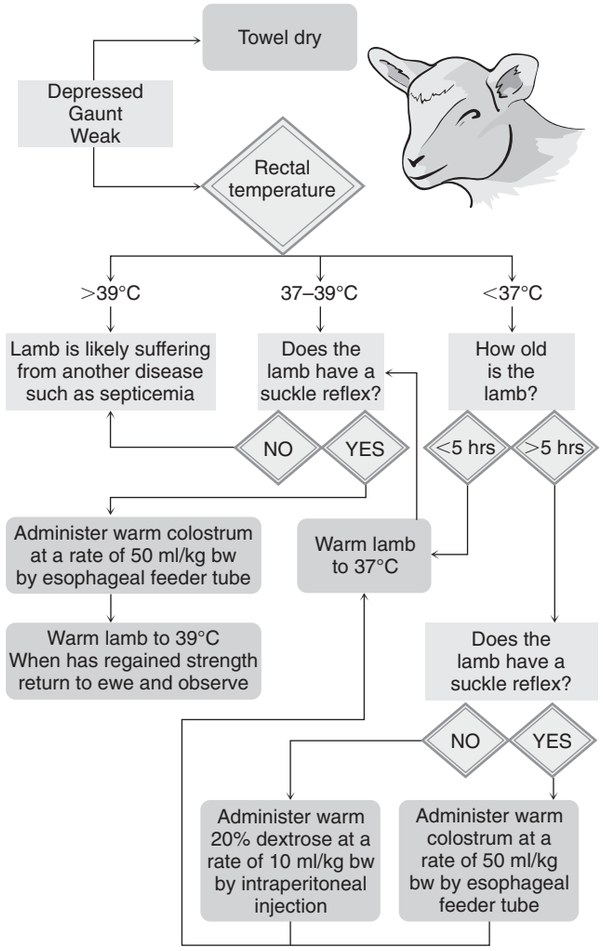

Fig. 91-5 Algorithm for treatment of hypothermia and hypoglycemia in neonatal lambs.

are hunched up and empty-appearing. If the problems are not detected early, these lambs will become recumbent. If in sternal position, the lamb may rise but appears depressed and slow to respond. Eventually, the lamb is laterally recumbent and comatose.

Figure 91-5 presents an algorithm for treatment for a lamb suspected of suffering from hypothermia or hypoglycemia. The client should be instructed in proper technique for tube feeding, with specific measures to avoid aspiration pneumonia: The client sits with the lamb on his or her lap, holding the lamb's head and neck gently in the nondominant arm. The tube is introduced slowly into the mouth and gently pushed toward the back of the throat. If the lamb sucks on the tube, a swallowing reflex is encouraged. Once the tube has been swallowed, the client can feel it pass down the lamb's neck in the esophagus. This maneuver should be practiced several times until the client can recognize the feel of the rubber end passing the fingers. If the tube enters the trachea, it cannot be felt externally. Premeasurement of the tube will help the client to determine if the tube has reached the abomasum. If the lamb coughs, the tube is withdrawn and another attempt is made. Colostrum can be fed by

syringe or squeeze bottle. The dose of 50 ml/kg of body weight should be fed slowly over 5 to 10 minutes, so that the abomasum does not overfill, leading to regurgitation. The client is instructed to kink the tube before withdrawal to prevent aspiration of milk.

Many ways to warm a lamb have been described. Immersion in warm water is the most rapid but requires that the client hold the lamb while in water and then dry it immediately, to avoid chilling from evaporation. Placement in a warming box in which the lamb lies on a grate that allows warm air to circulate around it generally works the best (Fig. 91-6). A household hair dryer works well for this purpose. Air temperature should not exceed 41° C. A warming pad will accomplish the same thing if the lamb also is covered with a blanket and turned frequently. Least efficient is use of a heat lamp, which offers only radiant heat to the exposed part of the lamb.

Lambs older than 5 hours of age require an energy source before warming. Failure to provide energy will result in central nervous system damage from hypoglycemia. If no suckle reflex is present, the lamb should have 20% dextrose administered into the intraperitoneal cavity. This procedure begins with drawing up 20 ml of 50% dextrose into a 60-ml syringe and adding to it 30 ml of hot, boiled, clean water. The resultant solution should be at body temperature. Instillation into the peritoneal cavity is accomplished by hanging the lamb vertically and injecting just off midline distal to the umbilicus, using a sterile 20-gauge needle and administering 10 ml/kg of body weight. After administration, the lamb can be warmed.

Once a lamb has received treatment for hypothermia/hypoglycemia, it and its dam should be identified for more intense observation. Factors that put lambs at risk for hypothermia and hypoglycemia include the following:

- Poor maternal nutrition during gestation (reduces the number of cotyledons and placental weight; brown fat stores [perirenal and pericardiac] in fetus; lamb birth weight; mammary development and subsequent milk production; colostrum quality and quantity)
- Low birth weight (<3 kg), which is highly correlated with poor brown fat stores
- High birth weight (>5 kg), which is highly correlated with dystocia
- Trauma or hypoxia due to dystocia or prolonged birth, which may be evidenced by meconium staining
- Premature birth (<142 days' gestation)
- Mismothering due to inexperience (e.g., ewe lamb), illness, stealing by another ewe, lack of availability of claiming area, claiming of only the firstborn lamb
- Insufficient milk available as a result of mastitis, poor late-gestation nutrition, maedi-visna (ovine progressive pneumonia) or other illness, low body condition score, poor udder or teat conformation, wool-covered udder
- Poor environmental conditions at birth of the lambs, such as a cold temperature (<13° C if fleece is wet), drafty conditions, and dirty or wet bedding or ground

Two exceptionally high-risk periods have been recognized:

Birth to 5 hours, when the lamb is wet. Losses at this time are due primarily to hypothermia or are secondary to birth trauma.

5 hours to 7 days, when hypothermia most often is secondary to hypoglycemia. Weakness after 48 hours of age also may be complicated by diseases stemming from inadequate colostrum intake.

CONTROL OF IMPORTANT DISEASES OF NEONATAL LAMBS

Entropion

Entropion usually is apparent within the first 2 weeks of age and manifests as ocular discharge, blepharospasm,

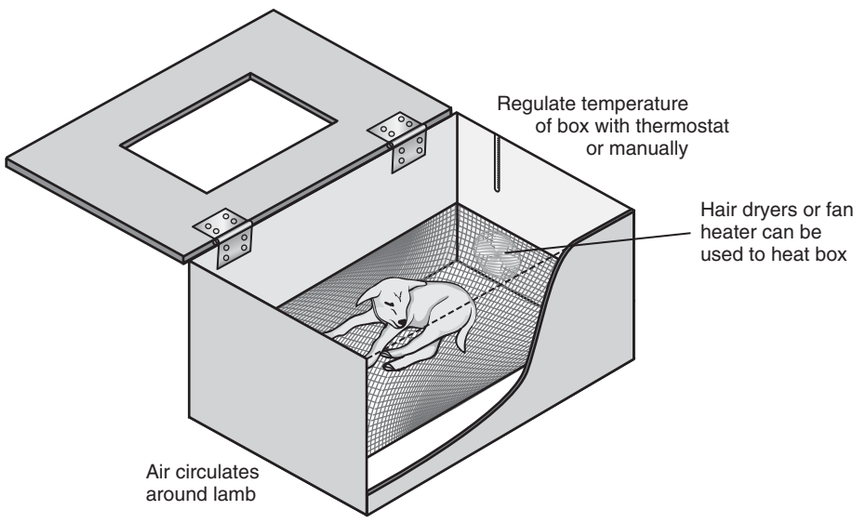

Fig. 91-6 Warming box for reviving hypothermic lambs.

corneal edema, and corneal ulceration due to irritation from inverted eyelids, usually the lower lid. Correction should be done immediately and may involve one of the following procedures: Mild cases may be resolved by manually everting the lid several times a day for a few days. Moderately severe cases may be resolved by an injection of penicillin into the palpebral conjunctiva. The resulting bleb should be sufficient to roll the eyelid out. Occasionally the procedure needs to be repeated in 5 to 7 days. Other, surgical-type methods include pinching a small amount of tissue into two wound clips placed parallel to the lower lid, crushing a fold of skin with hemostats parallel to the lower lid, and cutting a length of skin from below the lower lid and suturing it. With all of these latter methods, care must be taken to apprehend sufficient skin to allow proper eversion of the eyelid. Some evidence indicates that this is a heritable condition, so lambs with entropion should not be selected as replacements, particularly rams.

Diseases Due to *Clostridium* spp.

Tetanus, pulpy kidney, and enterotoxemia are diseases of neonatal lambs that can be easily prevented through a routine vaccination program with a multivalent vaccine. The vaccine should contain immunizing antigens to *Clostridium tetani, Clostridium perfringens* types C and D, *Clostridium septicum, Clostridium novyi* type B, and *Clostridium chauvoei*. Every replacement lamb should receive a primary series after 10 to 12 weeks of age and a booster at least every 12 months. To ensure optimal colostral protection to their lambs, the booster should be administered to pregnant ewes no later than 2 weeks before the first expected lambing date of their breeding group.

Coccidiosis

Clinical and subclinical coccidiosis is a major cause of poor growth in lambs. Management practices that will reduce environmental exposure to coccidial oocysts include use of properly designed feeders that do not allow for fecal contamination of the feed; provision of a clean bedding pack or slatted floors for nursing and growing lambs; and a raised water source. Thorough disinfection before lambing may reduce the level of coccidia in the environment, but adult ewes and then lambs quickly recontaminate the pens. It often is necessary to also use a drug that controls coccidia. Table 91-2 lists the commonly used preventive treatments for coccidiosis. Feed additives should be added to free-choice pelleted or texturized lamb rations to ensure adequate intakes. A prescription often is required for addition of these coccidiostats because of the variability among countries in licensing these drugs for use in sheep. Commercial cattle feeds or supplements with coccidiostats included should not be used because of the high risk of copper toxicity.

Table **91-2**

Drugs Commonly Used to Control Coccidiosis in Lambs

Commercial Name	Active Ingredient	Dose (BW/day)	Mode of Delivery	Comments
Various	Sulfadimidine-sulfamethazine	25–140 mg/kg	In water; 4 days on, 3 off, then repeat	Higher dose is used for treatment (4 days); toxic if treatment is prolonged; lower dose is used for prevention/control (long term)
	Sulfaquinoxaline	13 mg/kg	0.015% solution for 3–5 days	
Amprol*	Amprolium	20 mg/kg	5 days as a drench; 21 days in feed or water	Resistance reported; toxic at higher doses
Rumensin	Monensin	1 mg/kg	11 mg/kg in free-choice–fed or 22 mg/kg in limit-fed animals in complete feed for 6 to 8 weeks	Toxic if mixed incompletely or incorrectly
Bovatec	Lasalocid	1 mg/kg	36 mg/kg in free-choice–fed animals for 6 to 8 weeks	Moderate toxicity
Deccox 6% Premix	Decoquinate	0.5–1.0 mg/kg	1.5 kg of premix/ton of complete feed, or calculate according to known intakes for minimum of 75 days	Not toxic; can be added to mineral premix to prevent disease in pastured weaned lambs
Clinicox 0.5%	Diclazuril	1 mg/kg	Either 1 dose or 2 treatments 14 days apart at 3–4 weeks of age	Disease may occur despite treatment in highly challenging environmental conditions

*Merial, Duluth, GA.
BW, body weight.

Neonatal Diarrhea

Lambs are susceptible to the same infectious agents as those identified for calves. Generally, enteropathogenic *Escherichia coli* causes disease at 2 to 7 days of age. Rotavirus and coronavirus may cause diarrhea from 2 days to 3 weeks of age. Cryptosporidia most often affect lambs after 2 weeks of age. *Salmonella* spp. and *Giardia* are less common causes. If the flock is experiencing high morbidity from neonatal diarrhea during the lambing period, ewes that are still pregnant should be moved to a clean area for lambing. People managing the sick lambs should not handle newborn lambs without changing and washing their clothes in disinfectant soap. Vaccination of pregnant ewes with a bovine *E. coli* scour vaccine has been done, but a preferred approach is first to attempt to break the cycle by focusing on cleaning and disinfection of the lambing and lamb-rearing environments.

Pneumonia

The most common cause of pneumonia in neonatal lambs is *Mannheimia hemolytica*. Most often the strain involved is A2, with A1 being the most common strain in bovine pneumonia. Other agents that may be involved are *Mycoplasma ovipneumoniae* and *Pasteurella trehalosi*. Various viruses may play a role in increasing susceptibility of lambs to these disease agents. Environmental conditions, however, appear to be more important in determining the incidence of pneumonia in young lambs. Stocking density, humidity, diurnal or nocturnal temperature fluctuations, extreme heat and cold, frequency of air changes, ammonia levels, and dust all increase the stress on the respiratory system. In outbreaks of pneumonia, metaphylaxis has been used with success. Drugs that are licensed for use in sheep for pneumonia include short-acting oxytetracycline, tilmicosin (Micotil*), and ceftiofur (Excenel†). Other antimicrobials that may be effective but are not licensed for sheep include long-acting oxytetracycline and florfenicol (Nuflor‡).

Generally, antimicrobial resistance is not a reason for treatment failure. Most often the reason is failure to detect sick animals soon enough or not treating for long enough. It is important to train the client to observe nursing lambs carefully for depression. This should be done outside the pen so as to not arouse the lambs until identified for further clinical examination. Necropsy of dead lambs is a good way to determine the reason for treatment failure. A vaccine against *M. hemolytica* infection in sheep is not available in North America.

Suggested Reading

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*Provel, Elanco, Greenfield, IN.
†Pfizer, New York, NY.
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CHAPTER 92

Diseases of the Periparturient Ewe

MARIE BULGIN

Excluding the abortion diseases, the problems of the periparturient ewe often are metabolic and related to fetal number, nutrition, and management. With the advent of more prolific and productive sheep, the likelihood that the large animal practitioner will be called on to treat one of these various problems is high. As with many other diseases, the clinically ill animal may represent just the tip of the iceberg, and the challenge to the practitioner is determining whether the illness constitutes an isolated case or reflects a flockwide ailment.

THE "DOWNER" EWE

No doubt the most common problem seen by practitioners during the last trimester of pregnancy is the **"downer" ewe.** Although most owners and many practitioners associate this condition with pregnancy ketosis, a number of other conditions may manifest in the same way and should be ruled out before treatment for ketosis is initiated.

Outside of parturition itself, the last 3 to 4 weeks of the third trimester is the period of highest risk for the ewe as a number of changes occur. It is at this time that the fetus(es) has reached its period of maximal nutritional demand. The ewe's ruminal capacity is compromised as a result of abdominal space required for fetuses and the ewe herself becomes ponderous and unwieldy.

ACIDOSIS

To compensate for the reduced rumen volume, most owners begin the feeding of concentrates to the ewes during the last trimester, but because of disparity in gestation and fetal numbers, intake among the ewes may not be equal. Some owners inadvertently feed too much to begin with or increase the amount too rapidly. Occasionally, ewes escape and find their way to a concentrate source. Rarely, an alternative feed, high in fermentable carbohydrates, such as bread or tacos, may be offered in too great a quantity. The same amount of a cracked, rolled, or ground grain may be substituted for the whole grain, which ferments much more slowly.

Diagnosis

The ewe presents with signs similar to pregnancy ketosis. She will be anorexic, depressed, standing alone, and sometimes ataxic or down and reluctant to rise. Usually the history of being recently started on concentrates or of breaking into the granary will have to be extracted

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from the owner who generally is not aware of the significance of this information. The rectal temperature will be normal, diarrhea may or may not be present, ruminal sounds are absent and the ewe is depressed. Blood glucose and other blood chemistries will be in the normal range. Blood pH may be low normal to definitely acidotic coinciding with the intensity of the signs. A rumen pH below 5.5 is diagnostic. Rumen contents are usually easily extracted through the body wall of the left lower lumbar fossa, using a 35-cc syringe and 14G 1.5- to 2-inch needle. Rumen contents also can be extracted per os through a stomach tube. The pH is checked using a multicolor indicator pH paper.*

Treatment

Treatment is focused on returning blood and rumen pH to normal (7.4 and 6.5, respectively). Rumen pH varies according to the diet of the ewe. The rumen pH in ewes on forages ranges from 7.0 to 8, whereas that in ewes that have been on diets rich in carbohydrates for any length of time may be as low as 5.5. In a ewe on hay and a small amount of grain, rumen pH should be in the vicinity of 7.0. Intravenous administration of 2 to 4 L of bicarbonate (50 g/gal of water [313 mEq/L]) is in order if the blood pH is below 7.2; otherwise, oral medication usually is adequate. Calf electrolytes with bicarbonate such as Biolyte,† Calf Quencher,‡ Delyte H20K,§ Enterolyte,¶ Resorb,¶ or magnesium hydroxide** should be administered orally. Additional bicarbonate must not be added to electrolyte fluids, because sodium toxicity is likely to occur.

After administration of oral electrolytes, ruminal contents should again be tested for pH and further medication administered if necessary. Mineral oil can be given orally to promote rapid evacuation of the rumen, and an oral antibiotic such as neomycin or tetracycline given to reduce the activity of the fermenting bacteria. Flunixin meglumine†† injections (150–250 mg given intramuscularly) will make the animal more comfortable. Bicarbonate treatment usually has to be repeated in 2 to 4 hours, as determined by the amount of depression and results of another rumen tap.

A rumenotomy to remove the fermenting ruminal contents is the treatment of choice, particularly in severe cases; however, most owners balk at the cost of the surgery.

*pH paper, Schleicher & Schuell, Inc., Keene, NH.
†Biolyte, Electrolyte Formula for Scouring Calves, Pharmacia and Upjohn, Kalamazoo, MI.
‡Calf Quencher, Calf Electrolyte Formula, Vetco, Inc., St. Joseph, MO.
§D-Lyte H2O-K, Oral Electrolyte Supplement for Nonruminating Calves, Fort Dodge, Fort Dodge, IA.
¶Entrolyte HE, A Nutritional Supplement for Young Calves Pfizer Animal Health, Exton, PA.
¶ReSorb Oral Hydration, Electrolyte Product for Scouring Calves, Pfizer Animal Health, Exton, PA.
**Carmalax, Pfizer Animal Health, Exton, PA.
††Banamine Injectable Solution, Schering-Plough Animal Health Corp, Union, NJ.

Prevention

Prevention is focused on starting supplemental concentrate feeding slowly (no more than 1/4 pound per feeding), increasing the amount fed no sooner than every 3 to 4 days and no more than 1/4 pound at a time. Ground, cracked, or rolled grains should not be substituted for whole grain unless the amount is cut by half. About 24 inches of feed bunk space per unshorn adult ewe should be allowed. Ewes close to lambing, which are large and awkward, should be separated from the more mobile and agile ewes so that competition for the concentrates is relatively even and none are able to overeat.

TRAUMA

In cold climates, frozen or muddy ground causes footing to be unstable. When scrambling to reach the feed bunk, aggressive agile ewes may cause other ewes late in pregnancy, heavy with multiple fetuses, to slip and fall. Ruptured round ligaments, fractured pelvises, torn stifle ligaments, and fractured femurs are not uncommon. Obviously, these ewes will be found "down."

In such instances the ewe is bright and alert and will readily eat and drink. Her rectal temperature will be in the normal range. The complete blood count and blood chemistries are normal. A thorough examination of the rear legs, however, often will reveal crepitation or muscle fasciculation in the pelvic or thigh region.

Treatment

Pelvic fractures often heal on their own; however, the healed bone may be somewhat malaligned, causing narrowing of the pelvic opening with the risk of future dystocia. Fractures of the long bones are more difficult to manage. Use of Thomas splints usually is not feasible; the ewe generally is too clumsy and heavy to get up and walk with the device while she is still pregnant. The ewe typically will remain down for the remainder of her pregnancy. After lambing, if the tendons of the front limbs have not become contracted, some ewes become mobile, depending on the location and severity of the trauma.

Most owners will not agree to the expense of x-ray films or surgery. Many, however, are very willing to devote time to nursing care. Providing a place for the ewe unmolested by the rest of the flock, stretching the front legs daily, keeping her well bedded, and turning her once a day to prevent decubitus ulcers are recommended measures.

Prevention is similar to that for acidosis. Enough bunk space and the separation of ewes heavy with multiple fetuses will keep injuries at a minimum.

HYDROPS

Hydrops allantois and hydrops amnion are not unknown in the sheep. Generally, by the time the veterinarian sees the ewe, her abdomen has become so enlarged that the animal can no longer support her weight and becomes a "downer." Hydrops allantois is characterized by more

rapid enlargement of the abdomen than with hydrops amnion and is more likely to cause rupture of the prepubic tendon. Determining which one of the two is the cause of the enlargement is difficult, however, and probably makes little difference in the outcome.

Diagnosis

The most diagnostic sign of hydrops is the huge size of the abdomen. Ballottement performed through the body wall or ultrasound imaging will suggest excessive fluid. Often the ewe is depressed and anorexic, although if the condition has been present for only a short time she may still be alert and eating. Rectal temperature and blood parameters generally are normal.

Treatment

This condition carries a grave prognosis because the increasing size of the abdomen puts pressure on both the rumen and the diaphragm, reducing their capacity. Induction of parturition is in order, although dystocia with ensuing shock, dehydration, and death is not an uncommon outcome. Several injections of dexamethasone (10 mg, 24 hours apart) may have to be administered.

Dystocia occurs because the enlarged uterus tends to be atonic and probably is unable to push the fetus into the birth canal. There may be failure of the cervix to dilate, and the fetus may be hard to reach during attempts to help. The fetus(es) usually is small in size and often premature and born dead. Fetal ascites, anasarca, or maceration also can be observed and, in the bovine, usually is associated with hydrops allantois. Hypovolemic shock is the usual cause of death in the ewe, which dies during or just after parturition. Surviving ewes probably should be culled.

CALCIUM DEFICIENCY

In the bovine, calcium deficiency or milk fever is one of the most common problems of the period just before and after parturition, and low blood calcium is associated with many bovine problems after parturition. The ewe differs in that clinical hypocalcemia is relatively uncommon under normal conditions. When it is seen, however, it usually will be in large outbreaks. Up to 30% of ewes exposed to long-distance transport, experiencing sudden deprivation of food or feed supplying calcium, or grazing on oxalate-containing plants in late pregnancy may experience clinical hypocalcemia.

Because the calcium concentration is low in both grasses and concentrates, subclinical hypocalcemia may be an unrecognized factor in other periparturient problems when grass hay is used as winter feed.

Diagnosis

The clinical signs appear suddenly. Initially the ewe appears agitated or anxious, and tremors may be evident. As the problem progresses, the ewe goes down and may be found with the hind legs extended behind. The head may be stretched out or folded back into the flank as seen in the cow. The animal may be unresponsive and appear comatose. Regurgitation through the nose is not uncommon, and bloat occasionally may be present.

Response to intravenous calcium solutions designed for bovine milk fever is diagnostic but is confirmed by serum calcium determination, which will be below 4.5 mg/dl.

Treatment

Calcium gluconate solutions can be given subcutaneously. Calcium chloride solution, however, may cause skin irritation and occasionally causes sloughs, so intravenous administration is preferable. Calcium paste or gel* (1/5 or less of a tube) can be administered orally to animals that are still ambulatory but is very unpalatable and causes a burning sensation of the mucous membranes. Also, the pastes may not be very effective in animals that are mostly comatose.

Providing alfalfa hay is perhaps the best way to prevent further problems in the remaining nonaffected animals in the flock. Trace mineral-salt mixes with added limestone also can be made available.

PREGNANCY KETOSIS

Also known as pregnancy disease, pregnancy toxemia, and twinning disease, pregnancy ketosis generally is brought on by the competition for glucose between the ewe and her fetuses. This disease is the result of the negative energy balance secondary to an energy intake that is not sufficient to compensate for the increased energy demands of the fetus(es) in the third trimester. Thus, it is seen in multiparous animals on limited-energy diets, late in gestation.

Pathogenesis

The maintenance of adequate concentrations of glucose in the blood is critical to fetal growth, brain function, and milk production. Very little dietary glucose, however, is absorbed in the ruminant, because glucose is efficiently fermented by the ruminal flora. The greater part of ruminant energy needs is met by the short-chain fatty acids, acetate, propionate, and butyrate, produced by the ruminal flora. Blood glucose levels are largely maintained by gluconeogenesis, 85% of which takes place in the liver. Excess glucose is stored in the liver as glycogen, stores of which are small and inadequate for the ruminant's needs in late gestation.

In ruminant gluconeogenesis, the most important glucose precursor is propionate, which is derived from ruminal fermentation of high-carbohydrate-content feeds. Other glucose precursors include endogenous and exogenous amino acids, and glycerol and lactate are utilized to a much lesser degree. Ruminal fermentation of

*CMPK Gel nutritional supplement for dairy cattle, AgriLabs, St. Joseph, MO.

forage produces acetate, which is the precursor of long-chain fatty acids for fat storage.

A negative energy balance, induced by feed deprivation or a sudden demand for energy, results in a decreased ratio of insulin to glucagon, which, together with other hormones, activates lipases that convert tissue fat to free fatty acids (FFAs) and glycerol. This glycerol may be converted to glucose in the liver. The FFAs can be utilized by most body tissues, although the liver removes a large proportion of them. In the liver, the FFAs are used to produce energy (by means of the Krebs cycle) or are converted into ketones (acetone, acetoacetate, and β-hydroxybutyrate). The direction of FFA metabolism is dependent on availability of oxaloacetate. If production of ketones predominates, and levels increase above normal, appetite will be depressed. Thus, a negative energy balance in ruminants with ketonemia and appetite depression will, unless immediately rectified, tend to further reduce feed intake. This complex series of reactions culminates in an irreversible downward cycle known as **ovine pregnancy disease** or **pregnancy toxemia.**

Experimental models of ketoacidemia have shown that systemic hypertension can occur in pregnant ewes after as little as 24 hours of food deprivation.[2,3] Renal dysfunction begins with the onset of hypertension and results in as much as a 51% decrease in glomerular filtration rate. A great deal of variation has been reported in the ease with which the disease can be produced experimentally in sheep, but no apparent breed disposition has been confirmed. The variation apparently is dependent on the metabolic efficiency of the liver.[2] An ineffective gluconeogenic response to the continued preferential demands for glucose predisposes ewes to this disease.

Risk Factors

Pregnancy toxemia can be a primary condition resulting from a combination of a fall in the plane of nutrition during the latter third of pregnancy and a sharp increase in energy needs. It can be precipitated by a short period of fasting associated with crutching, shearing, vaccinating, or transport. Stress of sudden winter storms, shearing during inclement weather, or absence of adequate shelter may be involved as well. Multiparous thin or aged ewes, very fat ewes, and confined ewes in their last trimester are those mostly at risk.

Secondary pregnancy toxemia may occur as a result of any other condition that restricts feed intake or increases energy needs, such as acidosis, water deprivation, hypocalcemia, footrot, pneumonia, heavy worm infestation, or the chronic wasting diseases.

Diagnosis

Early signs include anorexia, separation from the flock, depression, and sometimes blindness similar to that caused by thiamine deficiency. Tremors, chomping of the jaws, presence of foam at the mouth, a strong sweet smell of acetone on the breath, "star gazing," circling, ataxia, hind end weakness, head pressing, and muscle tremors tend to occur a day or two later. Recumbency and occasional convulsions occur within several days, and the ewe becomes profoundly depressed or comatose before dying several days later. Fetal death may occur and sometimes results in a transient recovery. Toxemia caused by the retention of the dead fetus, however, eventually will cause death of the ewe. The clinical course often is shorter in fat ewes. Affected ewes usually have difficulty in lambing, and the lambs may be dead.

Blood chemistry analysis usually, but not always, reveals hypoglycemia (<40 mg/dl), an elevated blood urea nitrogen (BUN) and serum aspartate transaminase (AST), and acidosis. Hypocalcemia also may be present. Results of assays for urinary and blood ketones will be positive, although any ewe with twins close to parturition will test positive for urinary ketones. As the disease progresses, blood glucose and cortisol levels may be elevated (to greater than 70 mg/dl and10 ng/ml, respectively) as a result of fetal death. Dehydration and renal failure may be apparent.

At necropsy, liver is pale, tan in color, and swollen and will float in water. Multiple late-term fetuses will be found.

Treatment

Early intervention may have a favorable result, but in general, response to treatment usually is poor, because the disease becomes irreversible in its later stages. For best results, blood chemistry and electrolyte analyses should be done and the animal treated accordingly.

Traditionally therapy has been targeted at providing an appropriate energy source and reducing the energy drain. Constant-drip 5% intravenous glucose is ideal but may not be practical. An intravenous catheter could be placed and the owner instructed on administration of 25 to 50 g of glucose (50–100 ml of 50% glucose) several times a day. In an attempt to provide oral glucose precursors, many small doses of propylene glycol (10 ml) given every 2 hours over the course of the day is preferable to one or two doses of no more than 30 ml per treatment two or three times a day. A 500-ml bottle of amino acid–vitamin–electrolyte solution* can be given in small doses over a 12-hour period as a drench or added to drinking water. Calf electrolytes for neonatal diarrhea made up according to directions and given orally twice daily for the first day will combat acidosis and dehydration. Some of the glucose in the preparations may even bypass the rumen. An oral calcium supplement or an oral gel may be helpful, because hypocalcemia can be present in some cases, particularly if grass hay has been the major source of feed. Calcium, too, probably will be more beneficial if given in small doses over the day, rather than as one or two larger doses. Sodium propionate can be given if a source can be found. The B vitamins, cyanocobalamin (vitamin B_{12}) and biotin, are particularly indicated as adjuncts to gluconeogenesis, and thiamine (vitamin B_1) may help preserve brain function. Insulin, 25 U of protamine zinc given once daily, may be helpful, as well as vitamin E and selenium (25 mg given intramuscularly).

*Double "A" Solution, Vetco, Inc, St. Joseph, MO.

Ewes suffering from pregnancy toxemia may benefit from dipyridamole,* a thromboxane A_2 inhibitor and vasodilator used in human medicine with aspirin to reduce platelet adhesion.[1] It also produces a dose-related decrease in systemic and coronary vascular resistance, leading to decrease in systemic blood pressure and increase in coronary blood flow in the dog. The onset of action takes about 24 minutes, and the effect persists for about 3 hours.[4]

Removal of the fetuses by means of cesarean section or induction of parturition with corticosteroids has met with some success if done in the early part of the disease. Intramuscular dexamethasone in a dose of 10 to 14 mg usually will initiate parturition within 24 hours. Occasionally, if the duration of the pregnancy is less than 140 days, a second dose will be required.

Prevention

Prevention centers on provision of adequate energy to late-term multiparous ewes. The practitioner should keep in mind that a ewe with a single fetus requires only 260 kcal/day at 140 days of gestation, whereas a ewe with triplets requires 570 kcal/day—a 220% increase.

Heavy, awkward, ponderous ewes should be separated from those due to lamb later and fed separately with plenty of feed bunk room. Generally, identifying these ewes can be done by eye during the last stages of gestation, but ultrasound scanning would allow them to be separated earlier, so that feed to single-parous ewes could be reduced, whereas that to multiparous ewes could be increased. A good-quality fine-stemmed hay should be saved for the latter group, and these ewes also should receive at least $^1/_4$ pound of concentrate per fetus. Forced exercise by enlarging the distance between feed and water could be instituted in some cases.

OTHER PROBLEMS SEEN BEFORE LAMBING

Ringwomb and Early Dilation Syndrome

Ringwomb is a condition of sheep in which the cervix does not dilate during parturition. The specific cause of ringwomb is not known, but a lack of release of hormones involved in softening the collagen of the cervix or a lack of response of the collagen in the cervix to hormonal stimulation may be involved.

Mineral deficiency, malpresentation, premature lambing, presence of dead lambs, and consumption of feedstuffs that contain estrogen have been eliminated as causes of ringwomb. The occurrence of ringwomb has not been correlated with breed of sheep, age, and body condition score; a genetic correlation, however, has been recognized.[5] The occurrence of ringwomb appears to run within bloodlines, and when these bloodlines are inbred, the frequency of ringwomb increases.

Ewes with ringwomb generally do not isolate themselves, have a decreased appetite, or show the other normal signs of first-stage labor. No swelling of the vulva, relaxation of the pelvic ligaments, swelling of the vulva, or dilatation of the cervix is observed. Usually the first indication that something is wrong is the protrusion of fetal membranes from the vagina. If the ewe is left unassisted, the lamb(s) will die from hypoxia, presumably as a result of separation of the placenta. Even after the fetus(es) has died, the ewe will require assistance for delivery of the dead lambs. If no help is forthcoming, autolysis of the fetus will occur, leading to toxemia, septicemia, and death of the ewe. Antibiotics should be administered immediately to ewes found after the fetus(es) has died, to prevent systemic infection.

Early dilation syndrome is similar to but considered to be separate from ringwomb. Assistance is required for lambing because of incomplete dilation of the cervix approximately 1 to 2 weeks before term. If the ewe is left unassisted during lambing, she may be found dead as a result of uterine prolapse or uterine tears with evisceration. These ewes typically present with placental membranes protruding from the vagina and little or no udder development. As with ringwomb, no correlation has been detected with breed, nutrition, or toxicology.

Treatment

Treatment for ewes that are suspected of having ringwomb and in which the lambs are still alive is a cesarean section. This usually will result in the delivery of healthy live lambs. Because the condition appears to be genetic, lambs should not be kept for breeding stock. The ewe probably should be culled, even though such ewes usually are not affected by ringwomb during consecutive seasons.

Manual dilatation of the cervix with removal of the lambs is another option and often the one resorted to by the producer. Calcium administration and oxytocin usually have not been helpful, although they can be tried. Working one and then two fingers, and so on, through the cervix will eventually allow the practitioner to get a hand though the cervix. This takes time and patience, because the cervix can tear and the practitioner may suddenly find the hand in the abdomen. Once the lamb is grasped and delivered to the now half-open cervix, the pressure on the inside seems to aid in its final opening, and the lamb can be removed. Common sequelae to these two conditions are retained membranes, uterine infection, and cervical scaring.

Prolapse of the Vagina

Risk factors for prolapse of the vagina include overweight, multiple fetuses, and high-fiber diets. In addition, coughing, straining and genetic predisposition all can contribute to vaginal prolapse in the pregnant ewe.

Diagnosis is confirmed by presence of the unmistakable red, round ball of variable size protruding from the vagina of the ewe.

Treatment ranges from suturing the vulva closed to using a "prolapse harness" or prolapse paddle to keep the

*Persantine, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT.

vagina inside the vulva. All of these various techniques are effective if straining or coughing is not involved in the problem.

If the problem is merely ruminal overfill, and the ewe is not straining, then reducing space-occupying fiber in the diet and replacing it with more energy-dense foodstuffs constitute appropriate management. High-carbohydrate feed must be increased slowly to prevent acidosis. Higher-quality hay can safely replace poorer-quality hay and may help the situation considerably. Removal of dusty feed and antibiotic treatment or treating for lungworms may help to reduce coughing.

A caudal epidural block using a 21- or 20-gauge $1^1/_2$-inch needle and 4 mg of Xylazine (0.2 ml of 20 mg/ml solution) plus 2 ml of 2% lidocaine works well for placing sutures in the vulva. After 10 minutes, a purse-string or several horizontal mattress sutures using umbilical tape can be placed to secure the prolapsed vagina inside the vulva. The sutures must be placed close to the wool, where the skin is strongest; alternatively, stents or buttons must be used to keep the suture from cutting through the tender skin around the vulva. The ewe must be monitored closely for labor, at which time the purse-string suture is cut to allow delivery of the lamb(s).

If straining is the main cause of the prolapse, its control is necessary for the successful management of the prolapse. Coccidia have been implicated in some cases of straining, in which instance treatment with sulfa drugs has been helpful. In most cases, however, the cause of the straining forever remains a mystery. If intramuscular flunixin meglumine injections (150–250 mg) and an initial lidocaine epidural block are not successful, alcohol epidural blocks using equal volumes of 2% lidocaine and 70% isopropyl or ethyl alcohol occasionally are performed as a last resort. The volume of lidocaine-alcohol to use is determined by the response to a previous injection of lidocaine only. The duration and degree of response to the alcohol epidural block are variable. The sequelae to an alcohol epidural may include permanent paralysis of the tail and incontinence. Overdosage can result in permanent paralysis.

When the control of straining is not very successful, another method that may help is the Minchev technique, whereby the dorsal vagina is sutured in place by passing a ligature through the dorsal vaginal wall to the outside of the gluteal area, lateral to the second or third sacral vertebra. Both sides of the anterior dorsal vagina are anchored using umbilical tape or other heavy suture material. Buttons are fastened to both the skin and the vaginal ends of the suture, to prevent the suture from cutting through. This method usually secures the vagina despite constant straining, and because the vaginal opening is not restricted, the ewe can lamb without constant observation. Usually enough adhesions are formed to keep the vagina in place even after the sutures are removed.

Because this condition usually recurs and probably has a strong genetic component, prevention requires that these animals and others of the same bloodline be culled. Close monitoring of the body condition score of the ewes in the flock and preventing them from becoming overweight also will help. This is particularly important for ewes that have not lambed previously.

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CHAPTER 93

Reproductive Health Management Programs

PAULA I. MENZIES

Throughout North America, many systems are used for managing sheep, from the traditional annual spring lambing with fall marketing of lambs off pasture, to managing several lambing groups per year, to marketing lambs year round. The basic principles for a reproductive health management program remain the same but need to be fine-tuned with each producer's limitations and goals in mind.

FACTORS TO CONSIDER IN DESIGNING A PROGRAM

- Types of products to be marketed
  - Meat (light lamb, heavy lamb, mutton)
  - Wool (fine wool, specialty spinner's wool)
  - Milk (length of lactation, seasonal production versus year-round production of milk)
- Purebred breeding stock
- Type of markets
- Specific local market (e.g., Easter "new crop" lambs)
- Processing plant's time constraints on cheese production
- Cash flow requirement (may dictate year-round production)
- Forward contracting constraints from regional abattoirs
- Seasonality of markets
- Relationship between lambing and marketing—how old are lambs when they reach market weight?
- Frequency of lambing
- Nutritional/housing/pasture constraints on lambing
  - Producer motivation and skills
  - Capable of managing a complicated management scheme
  - "Weekend" farmer interested in lifestyle farming

TYPES OF REPRODUCTIVE MANAGEMENT SYSTEMS

- Annual lambing in season (Fig. 93-1)
- Spring grass
- Early spring or late winter confinement lambing
- Annual lambing out-of-season
- Fall lambing
- Frequent or accelerating lambing
- Three lambings in 2 years (Fig. 93-2)
- Cornell Star (five lambings in 3 years) (Fig. 93-3)

SETTING GOALS AND MONITORING PRODUCTIVITY

The flock health cycle is composed of the following components:

- Monitoring on-farm productivity
- Goal setting
- Implementing strategies to achieve those goals
- Monitoring the response to the strategies and the effect of outside factors on flock productivity
- Setting goals again

It is a continuous process for which the backbone is monitoring and goal setting.

Guides for Goal Setting

- Regional production averages
- Breed production averages
- Best expected productivity for specific management system and breed
- Producer's past performance and analysis of limitations on productivity

Record-Keeping Systems

Monitoring requires a functional record-keeping system and the ability to easily turn the data into information useful in the monitoring and management of productivity and health. Examples of common sheep record-keeping systems include head counts at specific intervals (e.g., number at breeding, number of ewes lambing, number of lambs weaned or marked), lambing diaries, individual animal paper records, and computerized on-farm sheep management systems.

An ideal sheep record-keeping system should have the following characteristics:

- Flexible and powerful enough to suit the multitude of diverse management systems
- Easy to use
- Efficient, requiring only a few minutes a day to update
- Streamlined, minimizing the number of places the data need to be recorded
- Designed to produce usable action lists (e.g., ewes ready to lamb, lambs to be weighed, culling lists,

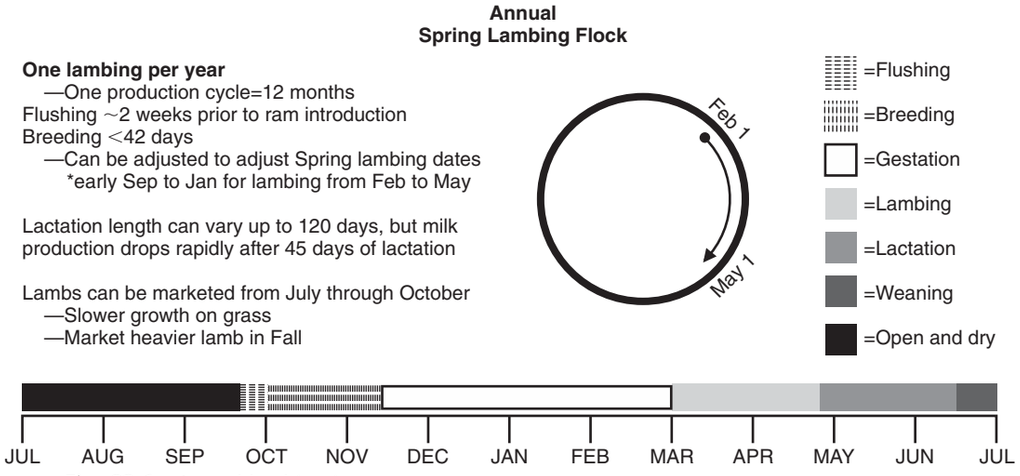

Fig. 93-1 Annual lambing in season.

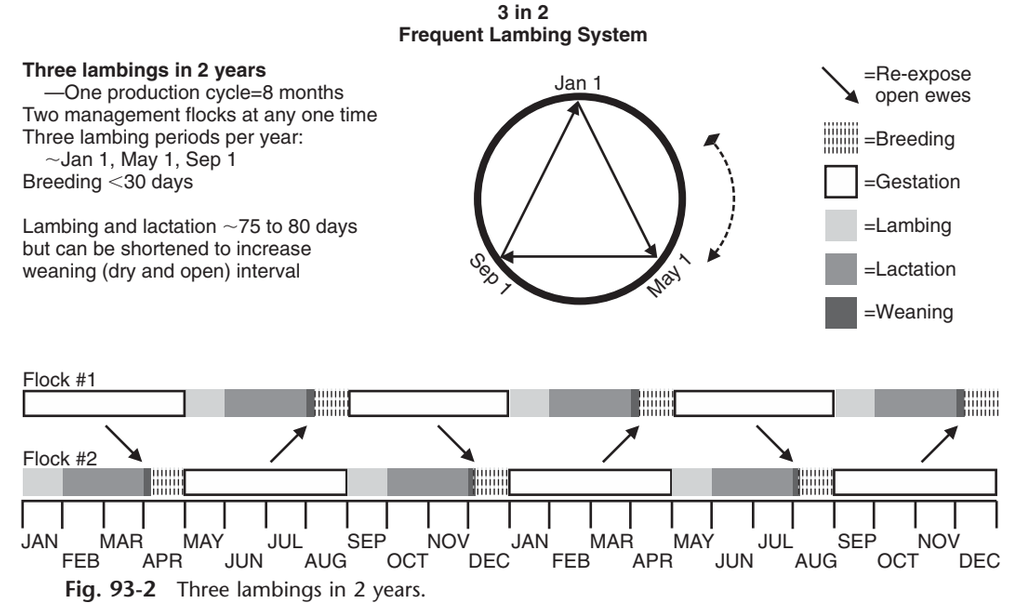

Fig. 93-2 Three lambings in 2 years.

animals to be vaccinated) that can be used in the barn, by the chute, or at the weigh scale
- Capable of monitoring reproduction, health and production performance and producing usable and meaningful analysis reports
- Compatible with regional, breed organization, or national production and health improvement schemes

It is unlikely with a flock size greater than 60 ewes that action lists and analysis reports can be produced easily and in a timely manner with any paper system. If individual animal records are to be kept, then it is necessary for the progressive producer to move to a computerized management system.

Information to Be Recorded

- Accurate numbers of animals at breeding, lambing, and weaning. Each ewe should be uniquely identified, and data pertaining to its performance are recorded at breeding, lambing, and weaning, as well as lamb or ewe morbidity, mortality, and culling
- Unique identification can be in the form of ear tags, tattoos, computer chips, or temporary paint branding
- Ram in and ram out dates, artificial insemination dates
- Ewes marked by ram marker harness during breeding. Color of marker can be changed every 14 days to record rebreedings

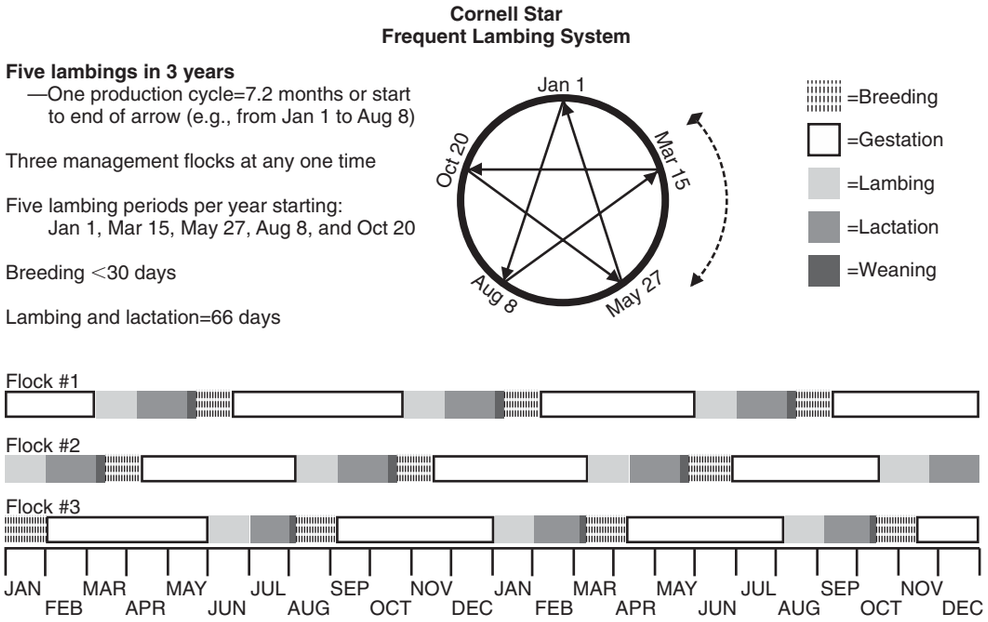

Fig. 93-3 The Cornell Star: Five lambings in 3 years.

- Observed abortions with diagnosis if available
- Lambing diary: ewe ID, lamb IDs, sex, status at birth (live/stillborn), assisted lambings, periparturient disease (e.g., pregnancy toxemia, vaginal prolapse, ring womb)
- Body condition score (BCS) at different stages of production
- Outcome of lamb rearing: died (age and diagnosis), marketed, or retained, or sold as breeding stock
- Fostered, artificial reared, supplemented on ewe
- Weaning weights (or weights recorded at other times if on national or regional record of performance [ROP] program)
- Prophylactic or preventive flock level treatments (e.g., anthelmintic dosing, vaccinations, hormones) and individual animal treatments
- Removals from the flock: involuntary culling including death, euthanasia, culling due to disease, and voluntary culling

MEASURES USED IN REPRODUCTIVE MANAGEMENT

Body Condition Score

BCS is a subjective evaluation of muscle development and fat cover and is a management tool rather than an outcome measure of productivity. Routine scoring of ewes and rams helps the producer make nutritional management decisions. If BCS is used correctly, many nutritional mistakes can be corrected before problems begin. See Figure 93-4 for an overview of a commonly used sheep scoring system. Sheep need to be handled in order to be accurately scored because of wool cover and because of breed differences in muscling and fat deposition. A maternal trait/dairy-type ewe (e.g., Finnish Landrace, Rideau, Romanov) will not carry the lumbar muscling of a meat trait breed (e.g., Suffolk, Texel) and may carry more internal fat. Practice and understanding of breed differences will allow a producer to accurately score both types. Ideally, one operator should perform all the scoring, because correlation between different scorers was poor, but with high intraobserver repeatability, in a 1998 study by Calavas and associates (see Bibliographic References at the end of the chapter). The sheep should be palpated over the spinous and transverse processes of the lumbar vertebrae, ribs, and shoulder and the muscles of the "twist" (hind legs and rump). This can be best done in a sheep race, with a drafting gate at the end to separate off the thin animals for special feeding. Goals for BCS and nutritional demands at the different stages of the production cycle are profiled in Figure 93-5.

Ram-to-Ewe Ratio

Factors used to determine the appropriate number of fertile breeding rams for the number of ewes include the age and experience of the ram (e.g., experienced versus maiden ram), ovulatory versus nonovulatory season, terrain (hill versus paddock), estrus synchronization in the ewe flock, length of ovulatory season of breeds being used, and fertility of those breeds during the nonovulatory season. Recommended ratios for common production situations are presented in Box 93-1.

Length of the Breeding and Lambing Period

The length of the breeding period—that is, period of exposure to the ram—will equal the length of the lambing season plus or minus approximately 3 days. Short, intense lambing seasons are useful for concentrating labor requirements, making more uniform feeding

SCORE 1
The sheep is thin and unthrifty with severe muscle wasting. The eye is sunken. The entire skeleton is easily palpated and sharp. The spinous processes of the lumbar vertebrae are prominent and when the sheep moves, the wool will part over the spine. All four fingers can easily press under the transverse processes and may lift the sheep from the ground in some cases. The sheep lacks energy and may lag behind the flock.

SCORE 2
The sheep appears thin yet strong. There is no muscle wasting but the entire skeleton is easily palpated. The transverse and spinous processes are slightly rounded but are still easy to discern. The eye is not sunken. Wool cover may mask the thin condition. The sheep will keep up with the flock but lacks sufficient reserves to withstand a prolonged period of underfeeding. Productive ewes may drop to this score after a heavy lactation.

SCORE 3
The sheep is thrifty with good muscle and some fat cover. The pelvis is still prominent but smooth. Boney prominences can be palpated with mild pressure but are smooth to the touch. A shorn sheep will have a smooth and healthy appearance. A productive ewe should be at this score before lambing and breeding.

SCORE 4
The shorn sheep appears bulky, particularly in the brisket area. The bony prominences cannot be felt easily except with pronounced pressure. The perineal and tail head area appear rounded. Breeding rams may reach this score before breeding. This is too heavy for a productive ewe.

SCORE 5
It is difficult for a productive sheep to achieve this score. Ewes that have been open for more than a year and have remained with productive ewes may get this fat. No skeletal features can be palpated on the body. The ridge along the spine actually dips in the center. The sheep has decreased exercise tolerance.

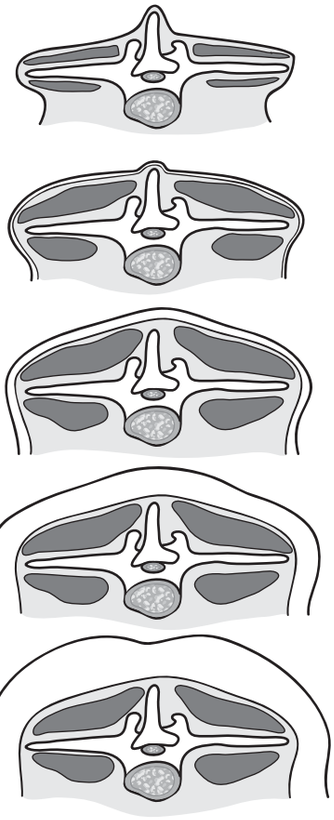

Fig. 93-4 Commonly used body condition scoring system for sheep.

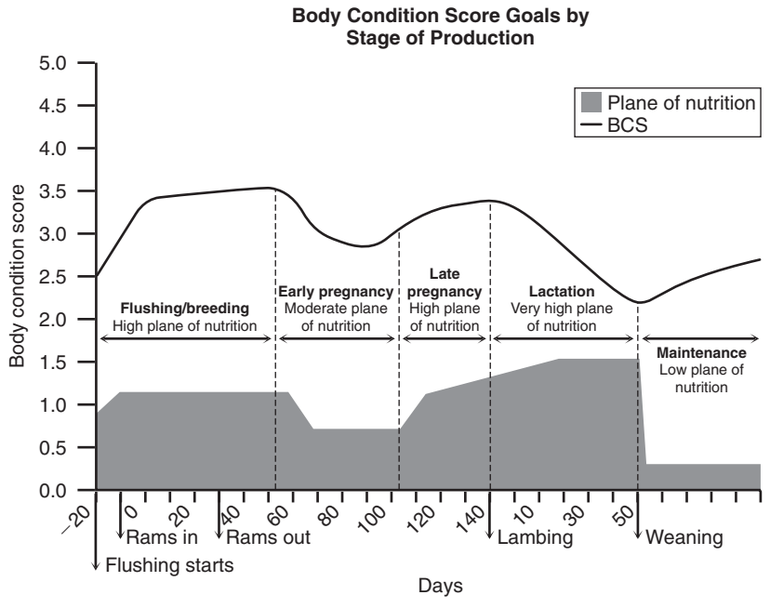

Fig. 93-5 Goals for body condition scores at different stages of production. (From *Body condition scoring of sheep.* Ontario Ministry of Agriculture and Food Factsheet, May 1985.)

practices, and improving lamb survival. In season, the normal duration of estrus in sheep is 17 days. So a 42-day breeding period is sufficient to achieve excellent conception rates in cycling ewes exposed to capable rams.

The practice of synchronization of estrus commonly is used to induce estrus during the transition period (3 to 6 weeks before the ovulatory breeding season), as well as out of season. Synchronization also can be used during the ovulatory season to shorten the breeding period even more. Figure 93-6 summarizes how the different methods of estrus synchronization can result in a tighter lambing period. If ewes are synchronized with sponges or prostaglandins in season, the breeding season will be 48 hours, optionally followed 10 to 12 days later with a clean-up ram for 6 days to catch repeating ewes. Out-of-season, synchronized ewes will have a breeding season of 48 hours.

Box **93-1**

Recommended Ram-to-Ewe Ratios

Mature ram in breeding paddock	1:40 ewes
Yearling ram in breeding paddock	1:25 ewes
Mature ram with maiden ewes	1:30 ewes
Mature ram on rough or barren terrain	1:30 ewes
Mature ram in flock synchronized with ram effect (transition)	1:20 to 1:25 ewes
Teaser ram used to synchronize flock (transition)	1:40 ewes
Mature ram in synchronized flock in season	1:15 to 1:20 ewes
Clean-up ram after synchronization (second cycle) in season	1:30 ewes
Mature ram in synchronized out-of-season flock	1:6 to 1:10 ewes

With some highly fertile rams on range conditions, ratios of up 1:80 to 1:100 have been used with success.

MEASURES OF PERFORMANCE: CALCULATION, INTERPRETATION, AND GOALS

Calculating Measures of Performance

Flock productivity is measured by calculating either ratios, proportions, or rates. A **ratio** compares measures that do not share any animals (e.g., ram-to-ewe ratio). For both **proportions** and **rates**, the **numerator** is the number of sheep that are affected (e.g., lambed, culled, died, treated) and the **denominator** is the number of sheep that are at risk of being affected (e.g., to lamb, the ewe must have been exposed to the ram). A rate also requires a specified time period (e.g., day, month, year, breeding season, birth to weaning) during which the animals were at risk. For example, *culling rate* is the proportion of sheep that were culled in 1 year (as defined in *Herd Health: Food Animal Production Medicine,* edited by O.M. Radostits, p. 53; see Bibliographic References).

Reproductive Measures of Performance

The following parameters measure different aspects of reproductive performance. Not all measures should be used in any one flock. A variety are presented here because it is important that parameters be clearly defined, as well as the method of calculation. The language of measuring reproductive performance in sheep is variable and not as well defined as with dairy, beef, and swine performance (as discussed in *Herd Health,* p 107).

The goals reported here will vary greatly depending on the management system and breeds used but are appropriate for most North American conditions. A schematic of these parameters is presented in Figure

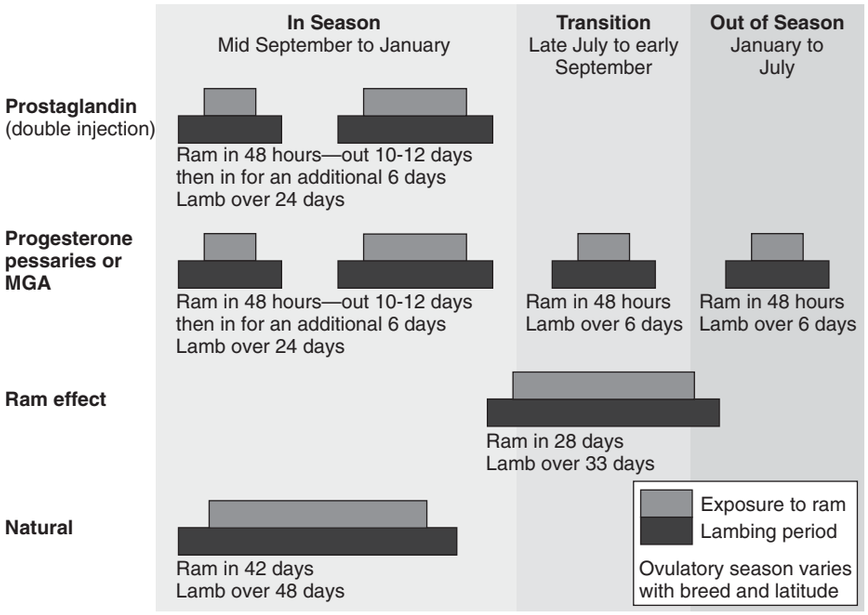

Fig. 93-6 Effect of estrus synchronization on length of lambing period. (Data from Henderson DC [ed]: *The veterinary book for sheep farmers.* Ipswich, UK: Farming Press Books, 1990, p 118.)

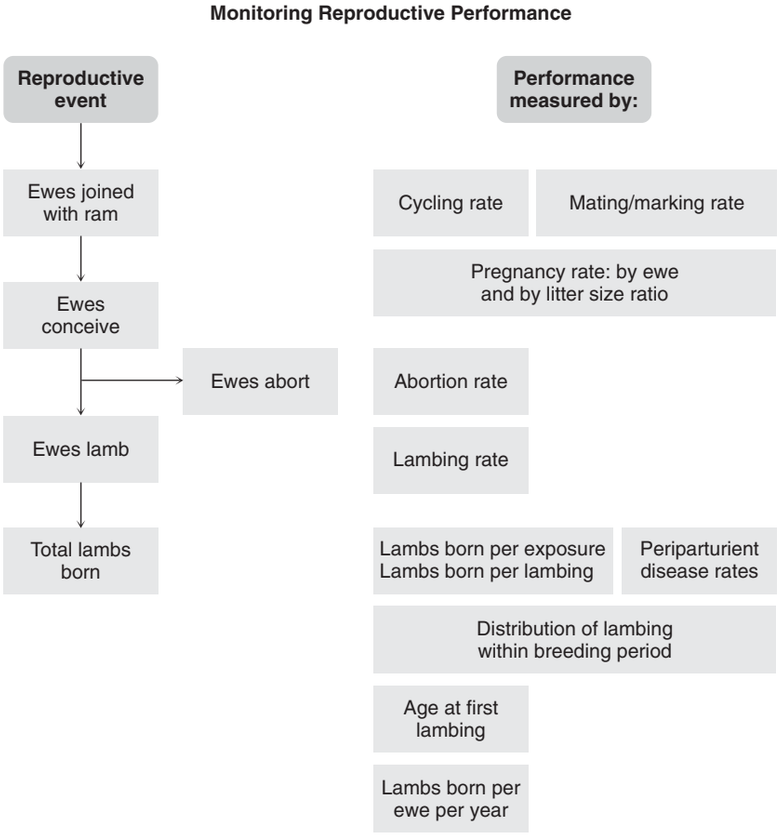

Fig. 93-7 Schematic of parameters used to measure reproductive performance.

93-7. Possible causes of reproductive failure are summarized in Box 93-2. Data from a 1996 survey conducted by the U.S. Department of Agriculture (USDA) (see Bibliographic References) are included where relevant. Table 93-1 summarizes data from Ontario flocks in the Sheep Flock Improvement Program and is useful for comparison with the USDA data.

1. **Distribution of lambing within the lambing period:** GOAL: For breeding during the ovulatory season, or if the ram effect is used during the transition period breeding, the ewe probably will have more than one opportunity to conceive. Approximately 75% of ewes should conceive to the first opportunity, 20% to the second opportunity, and less than 5% to the third. This is represented schematically in Figure 93-8.
2. **Cycling rate:** The percentage of ewes mated or marked by the ram during the first 14 days of the breeding period. GOAL: 70% of ewes in season. This is a measure of the serving capacity of the rams and how well the ewes are cycling. Failure of rams to mark the ewes with a marking harness may indicate problems with one or both groups.
3. **Mating rate:** The percentage of ewes mated or marked by the ram during the breeding period. GOAL: In season, a goal of 95% or greater is appropriate for mature ewes. Out of season, 70% is an achievable goal. The proportion for ewe lambs may be lower than this if they are 7 to 9 months of age and much lower if they are expected to breed at 7 months of age during the anovulatory season.
4. **Pregnancy rate:** The percentage of ewes exposed to the ram that are pregnant at pregnancy check per breeding period. GOALS: Mature ewes over two to three estrous cycles: greater than 95%; ewe lambs bred during the first year of life: greater than 75%; synchronized ewes in season, one breeding: greater than 70%; synchronized ewes out of season, one breeding: greater than 50%. Also termed **conception rate.**
5. **Abortion rate:** The percentage of ewes that have visible abortions before day 142 of gestation of ewes exposed to the ram, or pregnant at pregnancy check, per breeding period. GOAL: Visible abortion level should be less than 2%. Abortion rates of 5% to 7% may indicate enzootic disease. Some reabsorbed fetuses, early embryonic death, or missed abortions may be classified as conception failure in the ewe. The 1996 USDA survey indicates a mean abortion rate of 1.8% (±0.1).
6. **Lambing rate:** The percentage of ewes that lamb of those exposed to the ram per breeding period. GOAL:Mature ewes: greater than 90%; ewe lambs younger than 15 months of age at lambing: greater than 75%. This is a measure of fertility. Some producers and veterinarians use this term

Box **93-2**

Causes of Reproductive Failure in Sheep

Ram Failure

Failure to mate

Crayon failure
- Harness does not fit properly
- Wrong type of crayon

Lack of libido
- Disease
- Low BCS
- Photoperiodic effect

Reluctance to breed
- Infectious balanoposthitis ("pizzle rot")
- Contagious ecthyma of the penis or prepuce
- Lame (footrot, poor conformation)

Experience/age of male
- Too young or too old

Ram-to-ewe ratio
- Too few rams for season, terrain, breed, synchronization, age

Behavior
- Inter-ram aggression
- Ram bias against specific ewes
- Shy ram lamb

Failure to achieve pregnancy

May present as
- Returns to service
- Spread-out lambing period
- Late start to lambing
- Poor prolificacy in the ewes

Additional factors

Impaired fertility due to disease
- *Brucella ovis*
- *Histophilus ovis*
- *Actinobacillis seminis*
- Chorioptic mange of the scrotum
- Infertility after a fever

Impaired fertility due to injury
- Excessive heat
- Excessive cold
- Inguinal hernia
- Hematoma of scrotal sac

Impaired fertility due to environmental hormone disrupters (e.g., phytoestrogens or other toxins)

Testicular circumference too small because of age, season, genetics, disease

Abnormality of the penis due to congenital defect (anatomic) or trauma

Sperm abnormalities

Ewe Failure

Failure to be mated

Pregnancy

Anovulatory season
- May be more pronounced in ewe lambs

Prepubertal (age, breed, nutrition, time of year)

Failure of the synchronization program
- Improper feeding of MGA
- Pessary fallout
- Insufficient eCG dose or failure to give eCG
- Prostaglandins in anovulatory season

Environmental hormone disruptors
- Some clovers
- Toxins

Nutritional
- Thin or very fat

Lactation or recent weaning

Behavior
- Dominant ewe preoccupies ram
- Shy maiden ewes

Long tails physically impede breeding

Pseudohermaphrodite or intersex or freemartin

Failure to conceive or maintain pregnancy

May present as repeated marking if more than one opportunity to breed is provided

Problems with synchronization program
- Rams joined too early with ewes
- Too low a dose of eCG

Previous damage to reproductive tract

Early embryonic death from various causes
- Selenium deficiency
- Specific abortion diseases (border disease, chlamydiosis, toxoplasmosis)
- Vaginitis due to poor pessary hygiene
- Stress, e.g., run by predators, shipping
- Heat shock in early pregnancy
- High-level soluble protein in forage leading to high urea nitrogen levels in blood/tissues

Poor prolificacy

Insufficient energy at breeding (flushing, BCS)

Immaturity or old age

Seasonality

Insufficient dose of eCG

Genetics or breed of female

Any of above causes of early embryonic death

Severe nutritional deficiency after breeding

Also check for sufficiency of ram power

BCS, body condition score; eCG, equine chorionic gonadotropin; MGA, megestrol acetate.

incorrectly to state the number of lambs born to those ewes lambing. It is important that this term be clearly defined, similar to the cow-calf calving rate, to avoid misinterpretation.

7. **Lambs born per ewe:** This can be calculated in any of several different ways, each one giving a slightly different view of reproductive success. All lambs born at term should be included in the numerator, including stillbirths.

   Lambs born per ewe by breeding group: A *breeding group* is defined as a group of ewes managed similarly (e.g., same synchronization program, same breeding paddock) exposed (joined) to a ram(s) over a defined period—that

Table **93-1**

Summary of Annual Flock Productivity: Ontario, 1998–2000*

	YEAR		
Flock Characteristic	**1998**	**1999**	**2000**
Producers†	130	133	115
Breeding ewes	8,694	9,064	8,699
Lambings	9,717	10,296	9,730
Lambing interval (days)‡	354	333	330
Lambs born/lambing§	1.8	1.9	1.9
Ratio of singles-twins-triplets-quadruplets (%)	20.1:51.9:21.4:6.7	18.8:50.1:23.1:8.0	19.1:48.6:23.9:8.4
Lambs weaned/ewe lambing	1.6	1.6	1.7
Calculated total lamb mortality rate (%)¶	11.1	15.8	10.5
% of lambs dying at birth–10 days	5.6	5.8	5.5
% of lambs dying at 10–50 days	2.0	2.2	1.9
% of lambs dying at 50–100 days	0.7	1.3	0.9
Average 50-day adjusted weight (kg)¶	23.7	24.0	23.7
Average adjusted daily gain to 100 days (kg)¶	0.32	0.32	0.32

*Flocks enrolled in the Ontario Sheep Flock Improvement Program, as reported by the Ontario Ministry of Agriculture and Food, Guelph, Ontario, Canada.
†Twenty-eight breeds of sheep are represented by these data. The most common breeds are Polled Dorset, Rideau, Suffolk, and cross-bred.
‡The lambing interval of less than 365 days reflects the presence of flocks on accelerated lambing systems.
§All statistics are calculated by lambing, not by exposure or by year.
¶Lamb mortality rate by age is reported only if lamb losses were coded by the producer. Calculated lamb mortality rate was derived using the formula [(lambs born per ewe – lambs weaned per ewe)/(lambs born per ewe)] ×100% and probably is a better reflection of true lamb mortality rates.
¶Weights are adjusted by parity of the ewe (5 years), sex of the lamb (ram lamb), litter size (single), and age of the lamb (to 50 or 100 days of age).

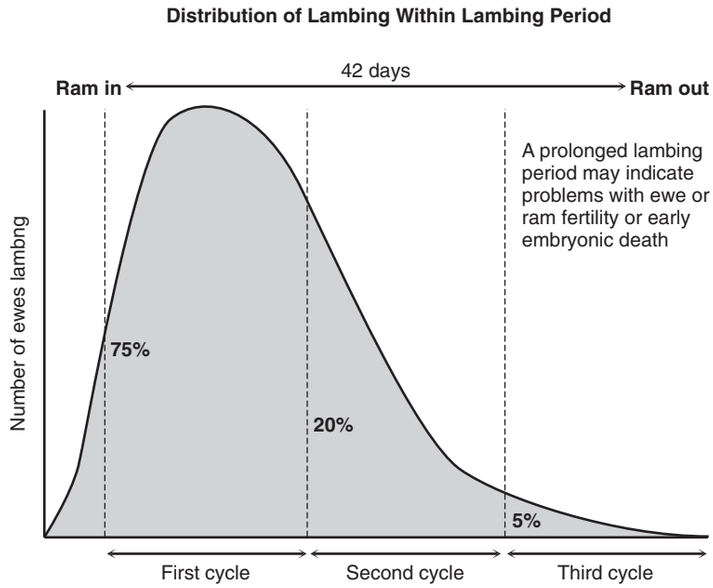

Fig. 93-8 Distribution of lambing within the lambing period.

is, with specific "ram in" and "ram out" dates. These can be expressed as a proportion (in decimal fraction form) or as a percentage (the proportion ×100%).

Number of lambs born per ewe lambing: This calculation is a measure not of fertility but of prolificacy. It often is termed **drop rate**, **litter size**, and (incorrectly) "lambing rate." It is one of the most often quoted measures of reproductive performance but can give a biased view of reproductive success because it ignores those ewes that did not conceive. GOALS: Range flocks: 1.3 to 1.6; prolific meat breeds under intensive management: 2.0 to 2.2.

Number of lambs born per ewe exposed to ram (reproductive rate): This calculation includes all ewes exposed to the ram in the denominator and therefore is a total measure of fertility, fecundity, and ability to maintain pregnancy in that breeding group. It sometimes is termed **reproductive**

rate because it reflects total reproductive performance for that breeding period. Producers should use this calculation as a critical assessment of the flock's or breeding group's reproductive performance. GOALS: Range flocks on rough terrain: 1.2 to 1.5; prolific meat breeds under intensive management: 1.8 to 2.2.

Number of lambs born alive per ewe exposed to the ram: This number subtracts stillborn lambs, which often are lost as a result of poor lambing management. GOALS: The 1996 USDA study indicates that of flocks surveyed, the mean number of lambs born alive was 1.213 (±0.012), with the highest numbers reported in flocks of less than 50 ewes (1.38 ± 0.014) and the lowest in flocks of more than 1000 ewes (1.08 ± 0.021). Producers may be tempted to count only those lambs born alive, but if stillborn lambs are ignored, this may give a biased view of reproductive success.

Distribution of litter size

Ratio of single births to twin births to triplet-plus births: Instead of calculating lambs born per ewe, some producers prefer to examine prolificacy by comparing the ratio of litter sizes. This measure of prolificacy can be calculated at lambing or, if available by real-time ultrasound using a sector scanner for fetal counting. GOAL: Optimizing the number of twins often is preferred, so that a reasonable goal for a moderately prolific flock might be 15%: 60%:15%, which would give a value of lambs born per ewe lambing of 1.8.

Lambs born per ewe by year: Although most flocks lamb only annually, many producers have switched to an accelerated (frequent) lambing system, with three lambings in 2 years being the most popular of these. It is important to analyze productivity by breeding group to ascertain problems with specific seasons, synchronization programs, or breeds, but producers also may opt to analyze the flock's reproductive performance by year in order to assess the overall performance of the accelerated lambing program. Total productivity by year should be significantly superior to annual lambing, although fertility may be somewhat sacrificed within breeding group, particularly outside of the ovulatory season.

Number of lambs born per ewe per year: Generally the denominator is all ewes that at any time during the year were part of the breeding flock (i.e., exposed to the ram), regardless of whether the exposure resulted in a pregnancy or whether the ewe was culled before the end of the year. Each ewe, however, is included only once in the denominator, even if she was exposed to the ram more than once. It is possible in some accelerated lambing systems for this statistic to be greater than 3 lambs born/ewe/year.

Number of lambs born per exposure per year: This is seldom calculated and is of use only in some accelerated lambing flocks. It would include all exposures and give limited additional information on the overall fertility and fecundity of the flock. Analysis by breeding group usually is of greater value.

Lifetime lamb production

Reproductive index: The total lifetime lamb production/(age of the ewe [years] – 1). This index includes all years in which a ewe was exposed to the ram for breeding, regardless of whether she lambed or not, and may be useful for indexing ewes within a flock, particularly with accelerated lambing systems (as reported by Burfening and colleagues in their 1993 article cited in the Bibliographic References).

8. **Periparturient disease measures:** Although not specifically related to reproduction, certain events, conditions, and diseases can cause greatly reduced productivity as a result of associated increases in ewe mortality rate and stillbirths.

Assisted lambings: The need for assistance in lambing will vary depending on the management conditions, but for an intensively managed prolific flock, less than 20% of lambings should be assisted, and for extensively managed, less prolific flocks, this level could be as low as 5%. Cesarean sections should be rare.

Vaginal prolapse: Preterm vaginal prolapse should be no more than 3% of the pregnant ewe flock. Higher levels should trigger an investigation of potential risk factors.

Pregnancy toxemia: Clinical pregnancy toxemia should occur in no more than 2% of pregnant ewes. Higher levels should trigger an investigation of β-hydroxybutyrate levels so that the nutritional status of the flock can be determined. In the 1996 USDA survey, 31.4% of producers surveyed felt moderate or high concern for this condition in their flock, and 19.2% reported that it was known to be present in their flocks within the previous 5 years. It was ranked 7th highest of 39 diseases listed.

Other diseases: The following diseases may occur sporadically or at significantly elevated levels:

- Ring womb, or failure of cervical dilation
- Premature cervical dilation, which may be associated with abortion diseases
- Hypocalcemia, which generally occurs 2 weeks ante partum
- Uterine inertia, which may be nutritional in origin

9. **Average age at first lambing:** The average age (in months) that ewe lamb's first lamb. GOALS: For meat breeds raised under intensive management, the goal

for breeding is 7 to 9 months of age so that ewe lambs first lamb at 12 to 15 months of age. Weight also is a consideration and varies with breed, but the ewe lamb should attain 70% of her mature weight at breeding, with a BCS of 3.0 to 3.5. Ewe lambs on extensive range conditions tend to be slower-maturing breeds (e.g., fine wool breed such as Merino or Rambouillet) and often are not exposed to the ram until the following year, to lamb at 2 years of age. This is not recommended for intensively reared ewe lambs for economic and ewe performance reasons. Age at first breeding will reflect voluntary management decisions to expose the ewe lambs to a ram. Age at first lambing also will reflect the breeding success of that exposure.

10. **Lambing interval:** The average days between lambings for the flock. GOAL: For once/year lambing flocks: 380 days; for flocks on accelerated lambing programs such as the "three in two," lambing interval should be closer to 260 to 280 days. This is a very historical measure of reproductive performance that is inherently biased because it includes only ewes with two lambings and does not include ewes that are culled as a result of poor reproductive performance or ewe lambs that fail to conceive.
11. **Lamb crops/ewe/year:** The number of lambings in a calendar year divided by the total number of ewes exposed to the ram during that year. GOALS: For once-a-year lambing flocks, 0.90 to 0.95; for accelerated lambing systems, 1.2 ("three in two") and 1.5 (the Cornell Star). This measure is useful only for sheep enterprises practicing accelerated lambing. All ewes that were exposed to the ram (i.e., at risk of lambing) should be included in the denominator, but only once.

Nonreproductive Measures of Performance

1. **Stillbirth rate:** The percentage of lambs found stillborn of the total number of lambs born per lambing period. GOALS: An appropriate goal would be less than 2% for less prolific flocks and less than 5% for highly prolific flocks. A stillborn lamb usually is one that is never identified as being alive at birth. In fact, the lamb may have lived for a short time, but death within 5 hours of birth is caused almost exclusively by parturient factors. Factors affecting stillbirth rate include dystocia (related to maternofetal disproportion, malpresentation, ringwomb, assistance), low lamb birth weight (less than 3 kg), intensity of observation of lambing, and exposure to abortion agents. Death after 5 hours may be related to environment or mismothering and therefore should not be counted as a stillbirth. The 1996 USDA survey reports stillbirth rate as a proportion of lambs born/ewe exposed to the ram. The value reported is 4% (±0.1%), for a ratio of 0.04 lamb born dead to 1.213 lambs born alive, or 3.3% of all lambs born.
2. **Preweaning mortality rate:** The percentage of lambs dying before weaning out of the total number of lambs born alive (or alive at 24 hours of age). GOAL: An appropriate goal for the total time period, as defined, would be less than 5%. Weaning occurs at various times depending on the management but usually occurs at 50 to 70 days of age. Many investigators studying perinatal lamb mortality (from birth to 7 days of age) prefer breaking the neonatal period into several time periods, such as less than 5 hours, 5 to 48 hours, 2 to 7 days, 7 to 30 days, and 30 days to weaning. Age at death is helpful in trying to determine the management factors involved in the increased mortality. The 1996 USDA study reports a preweaning lamb mortality rate of 9.4% (±0.3%).
3. **Lambs weaned per ewe:** As with lambs born per ewe, this parameter can be calculated by ewe exposed to the ram, by ewe lambing, and by year. This statistic is more valuable for economic calculations (i.e., the number of lambs available for sale or as replacements) than as an aid to the producer in identifying management problems. Poor reproductive and lamb survival performance is better investigated using the previous parameters.
   Number of lambs weaned/total ewes exposed to ram: GOAL: Slightly less than for lambs born per ewe exposed, as a result of lamb mortality.
   Number of lambs weaned per ewe lambing: Another measure used in extensively managed range and hill flocks is the **lamb survival** or **marking %** and is calculated as the ratio of number of lambs that come off pasture to be taildocked or castrated to total number of ewes exposed to the ram. It is a gross measure of reproductive performance and lamb survival and, as mentioned earlier, is more useful in economic calculations.
4. **Cull rate, mortality rate, turnover rate:** *Cull rate* is calculated as follows: (number of sheep culled)/(average number of sheep in the flock over a 12-month period). *Mortality rate* is calculated as (number of sheep dying of all causes)/(average number of sheep in the flock over a 12-month period). *Turnover rate* is calculated as [(number of sheep culled) + (number of sheep dying)]/(average number of sheep in the flock). Average flock number is calculated by averaging the number of ewes in the flock at the beginning of the year and the number at the end of the year. GOALS: Yearly ewe mortality rate should be less than 5%; yearly rate of involuntary culling should be less than 10%. Total culling rate (voluntary plus involuntary) should be approximately 20% of the adult flock. Cull rate can be further broken down into two broad categories: *voluntary* (as from breeding sales, low production, genetic turnover, and age) and *involuntary* (as from mastitis, mismothering, reproductive failure, and specific diseases that preclude successful production). Voluntary cull rates can be quite variable, depending on the goals of the producer. For health management programs, the rate of involuntary culling and mortality should be investigated. The 1996 USDA survey found that 16.1% (±2.0%) of adult sheep were culled annually and that another

5.1% (±0.1%) of adult sheep die, for an annual turnover rate of 21.2%. Of those sheep that left the flock, 6.0% left because of failure to lamb, 0.5% because of failure to meet ram breeding soundness evaluation parameters, and 1.9% from other reproductive problems.

5. **Average daily gain (ADG):** Weight of lamb at a specific age divided by the number of days of age. Timing of this measurement varies, but it usually is done at weaning (50 to 60 days) or at market age (100 to 180 days). GOALS: Goals are breed and management system dependent. For example, Suffolk lambs on full feed may gain 0.55 kg/day but less than 0.35 kg/day on pasture. Various ROP programs have adjustment factors for age of ewe, sex of lamb, and litter size in order to compare lambs and ewes within peer groups. Information on ADG can be used for ascertaining the growth performance of the group. Suboptimal performance should initiate investigations into nutrition and diseases that limit growth.
6. **Weight of lambs produced (kg)/ewe or hectare and weight of wool produced (kg)/ewe or hectare:** The total weight in kg of lambs weaned or marketed per ewe exposed to the ram, or per hectare of land used to support the sheep enterprise.

These are very broad measures and are designed to track economic progress. Although useful for the producer to determine efficiency of production, they are difficult to use for investigating poor performance.

FLOCK REPRODUCTIVE HEALTH PROGRAM

The common components of the reproductive health management program can be divided into those based on the ewe's reproductive cycle, presented in Table 93-2, and those components biologically based on season, presented in Box 93-3. To fit the many diverse sheep management systems, the veterinarian and the producer must consider both areas together. For example, parasite control should be based on season but may coincide with some reproductive procedures (e.g., pregnancy diagnosis).

CONSIDERATIONS FOR ACCELERATED LAMBING PROGRAMS

Programs become more complicated when accelerated (frequent) lambing programs are being used. Issues that need to be considered include the following:

- The producer must be able to cope with more than one production group at a time and to accurately time events such as breeding dates, ration changes, vaccinations, and other health management measures. This requires a more sophisticated record-keeping system and individual animal identification.
- Ewes are bred at traditionally infertile times of the year. The producer must have advanced knowledge on how to manage out-of-season breeding programs.

Box **93-3**

Sheep Health Management Program Based on Season

Winter—Temperate Climate

Advise producer:

- Drench all sheep for parasites with a larvicidal drug once sheep are housed or frost in ground.
- Ensure supplementation of selenium, iodine, and vitamin E to all sheep.
- Housing should be available to meet space recommendations for the different production groups.
- In cold climates, shear ewes if in closed "warm" barn.

Spring

Advise producer:

- Treat for parasites 3 weeks after turnout onto pasture; repeat in 3–4 weeks.
- *Warm climates:* Monitor parasite load using fecal egg counts or mucous membrane color (FAMACHA system).
- Shear if sheep are in long fleece.
- Treat for ectoparasites if these constitute a problem.
- Predator control measures should be in place.

Summer

Monitor for gastrointestinal parasites in ewes and lambs in mid-July using fecal egg counts or FAMACHA systems. Drench as necessary.

Instruct client on methods to prevent development of anthelmintic resistance

If footrot is diagnosed, institute control measures and follow-up eradication measures when spread time has passed.

Advise producer:

- Wean lambs onto clean pasture, stubble field, hay field, or dry lot.
- Predator control measures should still be in place.
- Monitor pasture productivity and, in combination with reproduction-related management procedures, monitor BCS.

Autumn

Nutrition Flock Health Visit

This visit may be in conjunction with veterinary-related tasks that occur at midgestation, such as pregnancy diagnosis.

Current year's forages should be analyzed and rations balanced for the following production groups:

Rams

- Breeding and maintenance

Ewes

- Maintenance and midgestation
- Flushing/early gestation
- Late gestation (based on expected prolificacy)
- Early lactation for singles and multiples

Lambs

- Creep
- Feedlot (market lambs)
- Replacement ewe and ram lambs

Drench for parasites if required (repeat monitoring for fecal egg count if was high in July).

Give iodine supplementation if sheep are on goitrogenic brassica pastures (forage rape/turnip tops).

Predator and footrot control measures should still be in place.

BCS, body condition score.

Table **93-2**

Sheep Health Management Program Based on Reproductive Cycle

Stage	Veterinary Tasks	Advise Producers to Perform Tasks	
Prebreeding 60 days before teasing required	Vasectomize rams for teasers	Reproduction	Vasectomized rams are not to be used for heat detection until 60 days after surgery
Prebreeding 7 weeks before breeding	Ram breeding soundness evaluation *B. ovis* serologic testing for high risk areas	Reproduction	Appropriate ram-to-ewe ratio Cull inferior rams
		Nutrition/housing	Nutritional flushing of rams to achieve appropriate BCS (3.5–4.0) Protect rams from adverse environmental conditions that might adversely affect spermiogenesis
		Disease control	Cull rams with epididymitis or orchitis
Prebreeding 4 to 3 weeks before breeding	Advise on abortion control (vaccination) Advise on parasite control	Nutrition	Sort and flush thin ewes <2.5 BCS Continue flushing until 3 weeks after removal of ram
		Disease control	Sort and cull unsuitable ewes (low BCS, bad teeth, mastitis, other disease such as footrot) Vaccination for abortion diseases if appropriate (veterinary advice) Optional anthelmintic treatment
Prebreeding 2 weeks before breeding	Advise on estrus synchronization program	Reproduction	*Transitional period*: introduce teaser rams at 40:1 for 14 days before fertile ram introduction *Estrus synchronization program*: vaginal pessaries in or start on MGA
Breeding 2 days to 42 days		Reproduction	*Anovulatory or transition season with P_4 synchronization program*: inject eCG Introduce rams ~24–36 hr after removal of synchronization agent Apply marking harness and change color every 14 days Record new marks every 2 to 14 days
Postbreeding		Reproduction	Remove rams and introduce teaser rams with marking harness to mark ewes still cycling (at risk of being open)—optional
Midgestation 7 to 12 weeks after ram removal	Pregnancy diagnosis with real-time ultrasound examination Check udders and BCS in ewes Discuss nutritional management of late-gestation ewes	Reproduction	Cull open ewes or slip to next breeding group if on accelerated lambing program
		Disease control	Institute prophylactic antibiotics when appropriate for control of infectious abortion
		Nutrition	Sort ewes by BCS and fetal numbers If no pregnancy diagnosis done, sort ewes by BCS so thin ewes can be supplemented
Late gestation 6 to 4 weeks before start of lambing	Prescription coccidiostat for lamb creep Discuss vaccination program for disease control in ewes Check lambing supplies/ medicines, including those for treatment of hypothermia/ hypoglycemia Discuss treatment algorithms for common diseases	Lamb care	Prepare lambing and lamb rearing facilities including milk replacer and equipment Order lamb creep with coccidiostat

Table **93-2**

Sheep Health Management Program Based on Reproductive Cycle—cont'd

Stage	Veterinary Tasks	Advise Producers to Perform Tasks	
	Discuss dystocia management, lambing, and neonatal care Investigate abortions		
		Disease control	Shear for indoor lambing Crutch for cold lambing and if wool is long Vaccinate ewes for clostridial diseases and CLA (optional) Ensure adequate vitamin E and selenium, either as injectable or delivered in feed Prelambing anthelmintic use if in program
		Nutrition	Increase energy and density of ration to all ewes, more to thin and prolific ewes Ensure adequate trace minerals and vitamins in ration
Lambing and lactation	*For high morbidity/mortality rates*: Perform necropsies of dead lambs Review treatment protocols for hypothermia/hypoglycemia	Lamb care	Work with veterinarian to determine reason for high morbidity/mortality rates Determine where breakdown occurs in lambing and lamb management protocols For infectious disease, institute corrective measures
Weaning	Calculate reproductive, health, and lamb performance statistics Make recommendations for change	Productivity	Cull ewes with udder disease, poor reproductive performance, history of prolapsed vagina, poor lamb performance, or other production-limiting disease Select superior replacements

BCS, body condition score; CLA, caseous lymphadenitis; eCG, equine chorionic gonadotropin; MGA, megestrol acetate.

- Although accelerated lambing systems make better use of facilities in that they are used year round, it is important that the facilities be large and elaborate enough that sheep can be separated by reproductive status and nutritional needs.
- Good handling facilities become essential so that sheep can be sorted accurately.
- The owner must be able to manage more finely tuned rations. Under traditional systems, ewes may have up to 5 months to recover weight after weaning before being bred again. In accelerated lambing systems, ewes may be expected to be ready for breeding almost immediately after weaning. BCSs should not be lower than 2.5 at weaning and should be 3.0 at breeding. This requires more accurate feeding of lactating ewes.
- Heavy feeding of lactating ewes may put them at increased risk of mastitis, particularly at weaning. This requires careful weaning management and monitoring of udder involution.
- Disease control becomes more difficult because of the mixture of age and production groups, and there is no "down" time in the flock. Accordingly, contagious diseases such as neonatal diarrhea become more difficult to control.

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CHAPTER 94

Reproductive Physiology and Endocrinology of Boars

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Modern swine operation viability depends on herd productivity and profitability. Herd productivity generally is assessed by reviewing herd reproductive performance and, in particular, performance of females in the breeding herd. Although gilts and sows constitute well over 95% of the breeding herd, the fact that overall reproductive performance is shared equally with the boars should not be overlooked. For this reason it is important to understand the underlying determinants of reproductive performance as they relate to boars used for natural service and those used for artificial insemination (AI).

PREPUBERTAL DEVELOPMENT

The prenatal testis develops in the fetal abdomen, near the embryonic kidney, and migrates caudally through the inguinal rings as it passes into the scrotum. In the pig, both testes should be fully descended at birth. Failure of one or both testicles to enter the scrotum results in cryptorchidism, a condition in which the retained gonad is capable of full endocrine function, although spermatogenesis is impaired owing to the thermal environment. Sperm production appears to be closely correlated with the number of Sertoli cells present in the testis, with a fixed ratio of spermatids to Sertoli cells being reported.[1] Sertoli cell proliferation in pigs is marked by two distinct phases, the first encompassing the period between birth and 1 month of age, and the second between 3 and 4 months of age.[2] Depending on the animal's genetics, steroid production from the Leydig cells of the testis and its subsequent effect on behavior (e.g., mounting pen mate, aggressiveness) can be apparent as early as 1 month of age onward.

PUBERTAL CHANGES

Puberty can be thought of a complex process leading up to full sexual maturity and is accompanied by distinct changes in the physiology and anatomy of the reproductive system.[3] The penis of the prepubertal boar lacks a sigmoid flexure and is attached to the lining of the prepuce by the penile frenulum. Under androgen influence, penis growth occurs and the boar's physical mounting activity increases; these events lead to the development of the sigmoid flexure and frenulum breakdown, respectively. Boars as young as 4.5 to 5 months of age can have spermatozoa present in the epididymis, but adequate libido for mating and ejaculation generally is not displayed until after 5 months of age.[4] Age at attainment of puberty can vary greatly between breeds and genetic lines.

MATURE BOAR

Reproductive Anatomy

The boar has a fibroelastic penis with a sigmoid flexure along its shaft and a characteristic corkscrew-shaped glans penis (Fig. 94-1). The boar's penis is retracted into the prepuce while at rest. The boar's prepuce runs medially along the distal third of the abdomen, with its wider cranial portion tightly attached to the ventral abdomen suspending the narrower caudal portion of the prepuce. It is normal for boars to maintain the penis in a retracted state and to display a jerking motion of the penis or prepuce during urination. In the dorsal aspect of the cranial portion of the preputial cavity, the boar has a unique structure known as the preputial diverticulum (see Fig. 94-1). This cavity collects urine, semen, and other fluids and harbors a large bacterial population. Contraction of the cranial preputial muscles in concert with mounting activity appears to empty the contents of the preputial diverticulum onto the shaft of the penis, providing lubrication for intromission. The glabrous, nonpendulous scrotum of the boar is located immediately ventral to the anus and contains a prominent medial raphe. Variation between boars in scrotal attachment may predispose some boars to be more susceptible than others to disruptions of spermatogenesis during the hot season of the year, especially those boars with testes held close to the body wall.

The testes are the site of spermatogenesis and hormone production. Boar testis weight has been shown to increase most dramatically between 4 and 6 months of age, reaching mature size by 8 months.[4] A recent study using B-mode ultrasound examination to measure testis diameter in vivo confirmed that in crossbred boars a plateau in testis growth is achieved by 8 months of age, with only a 2-cm difference in testicular diameter for boars 8 months to 2 years old.[5] The testes should by symmetrical and relatively uniform in size and have a distinct firmness to digital palpation. The epididymis functions as a site of sperm transport, maturation, and storage. The caudae epididymidis are palpable as distinct nodules located on the dorsal aspect of the testis within the

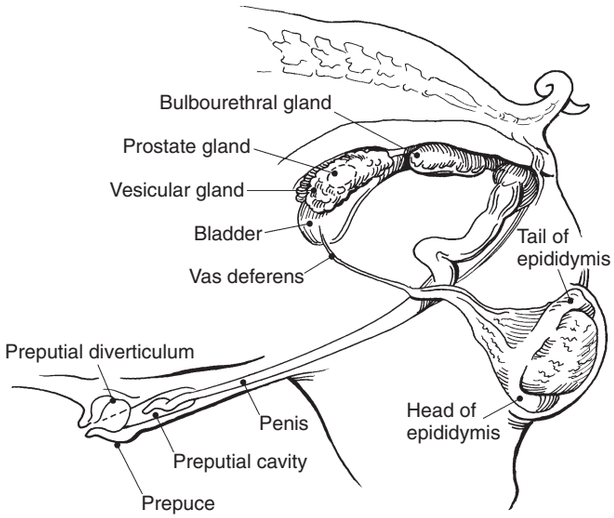

Fig. 94-1 Boar reproductive tract in situ.

scrotum. Proper location and identification of the caudae epididymidis are necessary in order to successfully perform epididectomy surgery in young pigs, a technique commonly used to create sterile teaser boars for estrus detection programs. The ductus deferens is the conduit used for passage of sperm from the cauda epididymidis to the pelvic urethra at the time of ejaculation. During vasectomy, sterility can be induced via ligation and segmental excision of a portion of the ductus deferens. The spermatic cord contains the ductus deferens and also serves as a vascular, lymphatic, and neural connection from the testis to the rest of the body. The scrotum, pampiniform plexus, and the cremaster muscle all aid in autonomic control of core testis temperature.

The boar reproductive tract contains accessory sex glands that contribute the seminal plasma portion of the semen (see Fig. 94-1). The vesicular glands are large, diffuse, lobulated structures, which contribute the bulk of the boar's ejaculate fluid volume. The prostate gland is much smaller relative to the other accessory glands and is located between the pelvic urethra and the ventrocaudal portion of the vesicular glands. The bulbourethral glands are large paired structures in the boar that straddle the internal pelvic inlet and produce the large gel fraction characteristic of boar semen.

Endocrinology

The reproductive endocrinology of the boar is discussed fully elsewhere[6]; a brief review is presented here. The pattern of gonadotropin-releasing hormone (GnRH) release from the hypothalamus stimulates the release of follicle-stimulating hormone (FSH) or luteinizing hormone (LH), or both, from the anterior pituitary gland. Sertoli cell function is regulated primarily by FSH. Sertoli cells are the only somatic cells found in the seminiferous epithelium and form the specialized blood-testis barrier. Sertoli cells are intimately involved in spermatogenesis, in addition to producing the hormones inhibin and estrogen. Inhibin provides the necessary feedback to the anterior pituitary to regulate FSH secretion. Estrogens influence sexual behavior and appear to control critical events during sperm maturation in the epididymis.[7] Leydig cells are the primary interstitial cells of the testis, and produce androgens under the influence of LH. Testosterone, the primary androgen produced, is necessary for normal sperm production and maintenance of the accessory sex glands and is involved with libido. Sexually excited boars chomp their jaws, eliciting the production of copious quantities of foamy saliva containing pheromones that stimulate sexual receptivity in estral gilts and sows. Active components in the saliva include the androgen metabolites 3α-androstenol and 5α-androstenone.

Spermatogenesis

Spermatogenesis is the process by which highly specialized spermatozoa are formed from primitive male germ cells. Spermatocytogenesis is the initial phase of spermatogenesis and consists of a series of mitotic divisions of the spermatogonia, ending in the production of primary spermatocytes. Primary spermatocytes undergo meiosis to produce haploid spermatids known as secondary spermatocytes. During spermiogenesis, haploid spermatids undergo dramatic morphologic transformations to become fully differentiated spermatozoa. After completion of spermatogenesis, spermiation ensues, which is the release of spermatozoa into the lumen of the seminiferous tubule. Sperm cells then undergo a final process of maturation in the epididymis in order to acquire motility and potential fertility. The cauda epididymidis also functions as the sperm storage reservoir until the time of ejaculation. Spermatogenesis takes approximately 35 days in the boar, with an additional 9 to 12 days needed for epididymal passage and maturation before becoming the fertile gametes emitted during ejaculation.[8–10] Therefore, it is not unrealistic to observe a 6- to 7-week lag time before a normal spermiogram can again be expected following a disruption in spermatogenesis.

Structure and Function of Spermatozoa

The sperm cell consists of the head and tail (consisting of a midpiece, the principal piece, and an endpiece). The head is 8 to 10 μm long by 4 to 4.5 μm in width and contains the haploid component of DNA in a highly condensed nucleus. Starting at the apex and encompassing roughly two thirds the length of the sperm head is a prominent acrosome containing the enzymes that aid the sperm cell in penetrating the surrounding mucoproteinaceous investments and intimate components of the oocyte. The midpiece is attached to the base of the head and makes up approximately 25% of the length of the tail. On light microscopy, the midpiece looks like a thickened portion of the tail. The midpiece contains a helical arrangement of mitochondria, which generate the energy required for the cell's motility. The midpiece ends bluntly, at which point the remainder of the tail continues on as the principal piece. The tail is very flexible and consists of nine outer dense fibrils (midpiece and principal piece),

an axoneme, and a fibrous sheath. The axoneme has nine pairs of microtubule doublets forming a ring around two central microtubules. Structural proteins link the outer microtubule doublets to the central pair of singlets, much like the spokes of a wheel; motor proteins attached to the outer ring of microtubules provide the force necessary to bend the tail to achieve motility. The axoneme continues through into the endpiece, the terminal 3 to 4 μm of the tail, which lacks the nine outer fibrils and the fibrous sheath.

Ejaculation

The boar is a pressure ejaculator, responding to the circumferential tightening of the sow's cervix around the glans penis. The gloved hand technique for semen collection takes advantage of this physiologic phenomenon by basically forming a fist around the glans penis, effectively "locking" the glans within a tightly gripped hand. The gloved hand technique is simple and quite effective, making the use of an artificial vagina outmoded and antiquated for porcine semen collection. Erection and ejaculation are primarily under the control of the autonomic nervous system, with ejaculation initiated by rhythmic contractions of smooth muscles lining the cauda epididymidis and ductus deferens. After initial thrusting motions, the boar will settle on a sow or collection dummy and proceed to ejaculate in phases for up to 5 minutes or more.

APPLIED REPRODUCTION IN THE BOAR

Prepubertal Boars

In general, crossbred boars reach sexual maturity at an earlier age than do purebred boars. Young boars that are reared in individual pens can experience a delay in puberty, poor sexual motivation, a decrease in mating performance, and increased locomotion difficulties compared with group-housed boars.[11] Interactions between pigs and their human caretakers are important in conditioning either approach or avoidance responses as the animal matures, which is an important point to remember in dealing with AI boars, which may fully mature at 225 kg or greater and will be handled in close quarters on a regular basis.[12,13] Developing boars may benefit from supplementary lighting that extends natural daylight to 15 hours per day by accelerating the onset of mating behavior[14]; however, sperm production, semen quality, and endogenous hormone profiles remain unaffected.[15–17] Although the detrimental effects of heat stress on mature boars are well documented,[18–20] it is not yet clear if high ambient temperatures during prepubertal development have long-lasting or permanent effects on sperm production or semen quality. Young boars have somewhat higher nutrient requirements than replacement gilts, but because growing boars are almost always reared under some method of performance testing for growth rate and feed efficiency, it is unlikely that their nutritional needs for sexual development or future reproductive capacity will go unmet. Exposure of prepubertal boars to commonly encountered levels of Zearalenone does not appear to have significant detrimental effects on future sperm production or semen quality.[21]

Postpubertal Boars

In general, crossbred boars tend to have heavier testes, increased sperm concentration, greater ejaculate volume, and improved semen quality in comparison with purebreds, although these differences may not be as dramatic as boars become older and full maturity is attained.[22] Significant differences in semen and reproductive characteristics (e.g., semen volume, sperm concentration, percentage of live spermatozoa, sperm motility, number of potential doses of semen) have been reported among breeds, although no single breed seems to stand out as excelling all categories.[23] Daily sperm output increases until purebred boars are about 18 months old, whereas crossbreds tend to peak earlier, at around 10 to 12 months of age.[4,5] Thereafter, sperm output remains fairly constant until it begins to decline at around 4 years of age, although a variety of factors may be involved in determining when senility sets in and sperm production drops off. Some studies have concluded that reproductive function in boars is stimulated by decreasing day length and inhibited by increasing day length, whereas others indicate that optimal sperm production is achieved when boars are exposed to a constant photoperiod of 10 hours per day versus 16 hours.[24,25]

Heat stress–induced reductions in semen quality are a major concern for boar study throughout many parts of the world. Considerable variation exists between boars in their individual susceptibility and response to heat stress, with genetically lean boars and those with testes held tight to the body wall at an increased risk. Variables such as the duration and intensity of the temperature elevation, relative humidity (RH), and ventilation in the housing area further complicate the issue. In general, exposure of boars to average daily ambient temperatures above 27°C and 60% RH poses a risk to semen quality. Much cooler temperatures, 15° to 20°C, appear optimal for boar comfort and semen quality. The detrimental effects of heat stress on the spermiogram begin to appear 7 to 14 days after insult. The observed increases in sperm abnormalities (e.g., head and tail abnormalities, cytoplasmic droplets), decreased motility, and decreased sperm output may be expected to continue for 5 to 8 weeks after the heat stress has been resolved. A similar pattern of events can be anticipated in boars that become systemically ill with concurrent pyrexia.

The ratio of boars to females in a natural service breeding program should be between 1:15 and 1:25. If the breeding program is 100% AI, then a ratio of 1:150 to 1:250 (average, 1:200) appears to be adequate. For efficient boar utilization, factors such as age, genetics, libido, season, and health should be considered in determining optimal collection and mating frequency. Suggested guidelines for boar usage are presented in Table 94-1. Increased collection frequency leads to lower sperm concentration and fewer sperm cells per ejaculate. Up to 60% of the sperm reserves stored in the cauda epididymidis are emptied in a single ejaculation, and it may take up to 8 days to completely replenish epididymal reserves once

Table **94-1**

Guidelines for Boar Usage Based on Type of Breeding Program

		NATURAL MATING PROGRAM		
Boar Age (mo)	AI Program Semen Collection Frequency*	Matings/Day	Total Matings/wk	Pen Breeding (No. of Females/mo)
6–8	1 time/week	1	4	<8
8–12	1–2 times/week	1	5–7	8–10
>13	More than 4 times/2 weeks	2 (spaced)	8–10	10–12

*Depending on boar libido.
AI, artificial insemination.
From Althouse GC: *Boar reproduction.* CAB International, in press.

exhausted.[9] Decreases in semen quality with negative impact on fertility are a concern if boars are overused,[4] and long-term high-ejaculation frequencies (e.g., 1 a day for 10 days) have been determined to induce potentially damaging biochemical changes in seminal plasma and sperm cells.[26] Overuse of boars younger than 8 months of age may result in unsatisfactory fertility as these boars mature, leading to premature culling. For AI boars, collection frequency is largely a matter of economic concern, with the cost of labor to collect the boar weighed against the expected sperm numbers to be obtained. Most commercial stud operations have found that intervals from three collections every 2 weeks up to two collections per week are optimal for their conditions (average: 1.35 times/week), with consideration given to genetic line and boar age.[27]

Hormone Therapy

In the United States, clinical use of reproductive hormones in boars is governed by the Extra-Label Drug Use provision of the Animal Medicinal Drug Use Clarification Act. The clinician is ultimately responsible for determining the legality of applying the therapeutics described here.

Although normal endocrine functioning is required for spermatogenesis, attempts to increase daily sperm production in mature boars through the use of exogenous gonadotropins have proved ineffective. Administration of GnRH, adrenocorticotropic hormone (ACTH), or human chorionic gonadotropin (hCG) can increase testosterone production in mature boars; however, whether a direct relationship exists between testosterone level and expression of sexual behavior remains a subject of controversy.

F-series prostaglandins have been promoted as useful in stimulating boar libido. Some boars appear to exhibit increased aggression toward the semen collection dummy after being given F-series prostaglandins. In boars, spontaneous erection and ejaculation have been reported within 10 minutes of intramuscular administration of 20 mg of F-series prostaglandin.[29] By contrast, other studies have failed to show a significant decrease in reaction time to mounting of an estrous sow or collection dummy after administration of 7.5 to 20 mg of dinoprost tromethamine (Lutalyse*) to boars.[30,31] No detrimental effects on semen quality or viability have been described in boars treated with $PGF_{2\alpha}$.

Pheromones are airborne chemical messengers that send signals between individual animals to elicit a response in other members of the species. The naturally occurring volatile pheromone androstenone, 5-androst-16-en-3-one, is bound by the protein pheromaxein found in boar saliva.[32] Aerosol cans of synthetic boar pheromones are marketed for use in AI programs and sometimes are used as an aid in training young boars to mount a collection dummy. Experienced AI boars generally will attempt to mount any stationary object roughly the size and shape of a sow. Young boars, however, often benefit from additional stimuli during the training process. A collection area that has fresh secretions from an older boar (i.e., saliva, urine, semen) can help stimulate mounting behavior in a new boar. Pheromone sprays probably function in a similar manner by mimicking the effects of saliva from a mature boar.

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CHAPTER 95

Infectious and Noninfectious Causes of Infertility in Boars

GARY C. ALTHOUSE

As in other livestock species, breeding soundness evaluations (BSEs) have value in identifying potentially subfertile or infertile boars. Unfortunately, BSEs have been and still are rarely performed on boars used in natural mating programs. With the recent exponential growth in the use of artificial insemination (AI) in the global swine industry, a more developed approach has evolved in evaluating the reproductive potential of an AI stud boar. Boars standing at an AI stud operation are routinely screened for selected pathogens that can reduce reproductive performance. Additionally, a large percentage of stud operations that collect and process semen from boars for AI routinely evaluate the motility, morphology, concentration, and volume of each ejaculate. The more recent incorporation of such screening protocols into swine breeding programs has greatly aided the industry in reducing the untoward effects of boar infertility or subfertility on herd reproductive performance.

Boar infertility is manifested in a herd as an elevated rate of normal returns to estrus, decreased litter size, increased litter scatter, or insufficient number of mated females per unit time. In retrospect, diagnosis of individual boar infertility is difficult to obtain because of the many confounding variables and management practices that can also influence herd reproduction. Accordingly, it is important that veterinarians and their producer clients take a proactive role in boar and ejaculate selection. One of the strengths of an AI program is the opportunity it provides to continuously monitor the boar's contribution to herd reproductive performance.

INFECTIOUS CAUSES OF BOAR INFERTILITY

Many of the pathogens that can cause boar infertility also have been found to use semen as a vector in their transmission. Infectious pathogens induce boar infertility through several general pathways. Certain infectious pathogens (e.g., *Brucella, Chlamydia,* Japanese B encephalitis virus [JEV], rubulavirus) can localize within the testicular parenchyma, with the ensuing inflammatory response disrupting spermatogenesis. Other infectious pathogens (e.g., swine influenza virus [SIV], *Mycoplasma,* the agent of erysipelas) indirectly disrupt spermatogenesis through the febrile response (with temperatures of greater than 40°F) associated with systemic illness. Finally, select infectious pathogens (e.g., the agent of classical swine fever, *Leptospira,* pseudorabies virus, parvovirus, porcine reproductive and respiratory syndrome [PRRS] virus) are shed in the semen of infected boars, causing disease in the female bred with the infected semen.

Owing to the devastating nature of infectious pathogens on herd reproductive performance, boar stud operations are constructed as independent facilities that preferably are sited away from any other livestock facility. Stringent biosecurity programs are followed to curtail the potential introduction of pathogens into the enclosed facility. Replacement boars, originating from selected pathogen-free herds, frequently undergo a 45-day (or longer) quarantine period. During quarantine, periodic serologic testing along with daily observation for clinical signs of disease is performed to minimize the introduction of disease to the resident stud group. If an animal exhibits clinical signs or has a positive test result for an unwanted disease, it is not uncommon for the farm to reject the entire group of replacement animals. Boar stud operations shoulder a great responsibility to maintain a disease-free herd so that economically devastating pathogens do not originate from them.

To elucidate the impact of infectious disease on boar stud productivity, a survey was conducted by Althouse in 2000 that encompassed 35 U.S. stud operations that stood 11,927 boars. A total of 25.7% (9 out of 35) of studs surveyed experienced a disease outbreak in the resident stud during 1999. Diagnosed causes included swine influenza [h1n1 or h3n2, or both—in most cases the specific type was not reported] (n = 6), PRRS (n = 2), mycoplasmosis (n = 1), salmonellosis (n = 1), and erysipelas (n = 1). In a typical disease outbreak, clinical signs were first observed approximately 4.6 days after the introduction of replacement boars into the resident stud group (n = 6 studs). Clinical signs usually were expressed by 26.7% of the boars standing at stud, with 89.2% fully recovering from any one incident. A negative effect on stud productivity (e.g., decreased semen quality) was observed an average of 32.4 days after onset of clinical signs.

SPECIFIC DISEASES

Porcine brucellosis is an infectious disease that can lead to swine infertility. Regulatory programs have been in place for several decades, effectively eliminating this disease from U.S. commercial herds. Feral hogs are considered a reservoir for this disease. Porcine brucellosis is

still prevalent and is considered a major cause of reproductive failure in swine of other countries (e.g., Africa, Asia, South America). In boars, *Brucella suis* (biovars 1, 2, and 3) initially invades regional lymph nodes, with ensuing bacteremia. This bacteremia leads to localization of *B. suis* in the genital organs, particularly those containing high levels of erythritol (e.g., the testis). A persistent orchitis or accessory sex gland infection ensues. *B. suis* is shed in the semen of infected boars, resulting in abortion and infertility storms. Diagnosis is by serologic testing. Depopulation of infected swine herds is recommended.

Leptospirosis is a common bacterial disease of swine that can cause reproductive loss. Multiple serovars of *Leptospira interrogans* (pomona, canicola, icterohemorrhagiae, grippotyphosa, muenchen, hardjo [sejroe], bratislava, tarassovi, australis) have been found to be involved in causing swine leptospirosis, with each serovar having a potentially different epidemiology. In boars, oftentimes leptospirosis infection is inapparent. Carrier animals or bulls with infected semen are responsible for disease spread. Screening or diagnosis of the disease in boars is by serologic testing. Effective commercial bacterins are available to prevent infection with this disease. These bacterins commonly are administered to all breeding stock according to label instructions. Antibiotics (e.g., tetracyclines, dihydrostreptomycin) can be administered to boars to reduce shedding of leptospires but may not eliminate the carrier state of the disease.

Chlamydial infections in boars have been associated with orchitis, epididymitis, urethritis, and reproductive disturbances. Weakness in newborn piglets and stillbirth can result from venereal transmission of *Chlamydia* organisms. The prevalence of chlamydial infections in swine herds is unknown, but serologic studies suggest that most herd infections are clinically inapparent. Diagnosis of chlamydial infections in swine herds as a cause of reproductive loss is uncommon.

Numerous viruses can affect boar fertility and may use semen as a transmission vector. Important pathogenic organisms in the global swine industry include pseudorabies virus (PRV), porcine parvovirus (PPV), and porcine reproductive and respiratory virus (PRRSV). Other viral pathogens of regional concern are hog cholera/classical swine fever virus, porcine rubulavirus, and JEV.

Pseudorabies, also known as Aujeszky's disease, is an important disease of swine worldwide. The disease, caused by a herpesvirus, is spread by contact, by aerosol transmission, transplacentally, or through excretion in the semen. Viral replication occurs in the genital tract. Semen quality in recently infected boars may be reduced indirectly because of fever. As with disease due to other herpesviruses, PRV infection can become latent, with recrudescence of the virus when the animal is under stress. Affected herds may be asymptomatic but often suffer reproductive failure, high neonatal mortality rates, and suboptimal growth performance. Commercially available vaccines control losses due to the clinical disease but do not prevent spread of the virus. Serologic screening of boar and biosecurity are essential to prevent PRV entrance into a swine herd. Economic losses were so great in the US that a national eradication program was justified. This current program involves test and removal, herd depopulation, or offspring segregation within infected herds.

PPV is a highly stable virus that is ubiquitous among swine throughout the world. Before preventive medicine programs, PPV was one of the most common causes of infectious abortion in swine. The most common routes of infection are oronasal and venereal. Naturally infected boars generally fail to show any clinical signs associated with infection but nevertheless can shed the PPV in semen. Embryonic and fetal death (including mummies and stillbirths) are common clinical signs observed in naive animals infected with PPV. Other clinical signs may include elevated rates of normal and delayed returns to service and abortion. Proper acclimatization of replacement animals, through vaccination and cull animal exposure, before introduction to the resident herd, has been quite successful in controlling this disease.

PRRSV, an arterivirus, has come to the forefront as one of the top etiologic agents of reproductive failure in swine today. Direct pig-to-pig contact seems to be a major route of PRRSV disease transmission, with infection also being able to occur by aerosol and through semen. Clinically infected herds experience reduced conception and farrowing rates, elevated neonatal mortality rates, and chronic respiratory disease with poor performance in growing swine. Symptomatic therapy appears to be only palliative. Vaccination appears to offer some control over the disease. In the absence of other significant endemic diseases, boars recently infected with PRRSV exhibit few clinical signs beyond those associated with a mild pyrexia. In the presence of endemic disease, PRRSV-infected boars generally exhibit pronounced clinical signs associated with the endemic disease, with disturbances in semen quality associated with the pyrexia. Excretion of PRRSV through semen appears to occur most frequently during the acute phases of the disease. Duration of excretion in semen appears to vary, with reported cases of prolonged or intermittent excretion well beyond 1 month after infection. Carrier states are believed to occur in some animals. Diagnosis of PRRSV disease in boars is based on serologic testing and screening of semen by polymerase chain reaction (PCR) assay.

The industry still struggles to define a program that provides effective control of PRRSV disease. Because of the inherent difficulties associated with endemic PRRSV infection, most boar stud operations typically strive to achieve a PRRSV-negative or -naive status. Many such operations apply a rigorous serologic and semen testing program during quarantine to minimize the chances of introducing PRRSV into the resident boar group. Accidental introduction of PRRSV into a boar stud group generally leads to its complete depopulation, followed by repopulation with naive animals. Boar vaccination with modified live virus attenuates may lead to shedding of the modified live virus in the semen; use of this semen may lead to seroconversion in the recipient animals, confounding future disease control strategies for the herd.

Hog cholera—classical swine fever—is a highly contagious, reportable disease of swine. Significant economic losses to the swine industry led to institution of a national program that resulted in its eradication in the

United States in 1976. Pigs appear to be the only natural host for the virus. Hog cholera virus, a pestivirus, is excreted with oronasal and lacrimal secretions and in urine, semen, and feces. Owing to the hardiness of the virus, mechanical vectors and garbage that contains pork products also appear to contribute to the spread of the disease. Acute infection induces a variety of clinical signs, usually leading to the death of the animal 10 to 20 days later. A 2000 report from Bouma and associates has demonstrated the regional spread of hog cholera through extended semen processed from recently infected hog cholera virus–infected boars. This possibility demonstrates the necessity for prudent quarantine and diagnosis for studs exhibiting clinical signs associated with infectious disease.

Porcine rubulavirus, a paramyxovirus, is the agent of blue eye disease, found in Central America, which can cause infertility in boars and sows. Central nervous system signs, corneal opacity, and high piglet mortality rates also can be observed in infected herds. Infected boars may show severe epididymo-orchitis and reduced semen quality, leading to temporary or permanent infertility. Diagnosis is based on clinical signs or with serologic testing, or both. Seropositive breeding stock should be culled.

Japanese B encephalitis is a mosquito-borne viral disease of swine that causes reproductive failure in several Asian countries. Summer infertility in boars appears to be associated with JEV, a togavirus. Experimental infections resulted in edematous and congested testes, epididymitis, reduced libido, and reduced semen quality. Infection can lead to temporary or permanent infertility. The virus can be shed in semen of infected boars, suggesting that semen can be a vector of disease transmission. Diagnosis of JEV disease in boars is through clinical signs or serologic testing, or both. Seropositive boars should be culled. Live attenuated vaccines have been used successfully to prevent infection in endemic areas.

NONINFECTIOUS CAUSES OF BOAR INFERTILITY

The most commonly observed cause of boar reproductive failure, infectious and noninfectious, appears to be heat stress–induced subfertility or infertility. Boars exposed to increased ambient temperatures (higher than 27°C and 50% relative humidity), or heat stress, exhibit a decrease in reproductive efficiency. More specifically, alterations in semen quality and libido are associated with heat stress in boars. Effects of heat stress on the spermiogram include decreased sperm motility, increased numbers of morphologically abnormal sperm, and a decrease in total sperm numbers. The negative effects of heat stress on the boar spermiogram are first evident starting approximately 2 weeks after initial heat stress exposure and can last for up to 5 to 8 weeks or longer, depending on the duration of the thermal insult. In some boars the changes remain unresolved; use of their semen in breeding programs will lead to decreased herd reproductive performance. Susceptibility to heat stress appears to be boar specific, with an as-yet undetermined genetic predilection. For optimal fertility, boars should be housed in a facility with a controlled environment. Environmental cooling (fans, water sprinklers, evaporative systems, geothermal cooling) is essential during hot weather to prevent heat stress in boars.

Cold stress–induced subfertility and infertility have been described in boars housed outside during winter conditions. Under severe circumstances, frostbite of the scrotum can result but does not appear to be necessary for the negative effects of cold stress to occur. Changes in the spermiogram include decreased sperm motility, increased numbers of morphologically abnormal sperm, and a decrease in total sperm numbers. In some situations, azoospermia may develop. Resolution of a short-term cold stress–induced infertility can take up to 2 months or longer. In some boars the changes go unresolved. Boars should be protected from extreme weather by use of insulated housing, adequate bedding, and proper nutrition.

Vaccine-induced subfertility and infertility also have been observed in boars. In the aforementioned 2000 survey, 45.7% (16 of 35) of the stud operations reported negative effects of preventive vaccination on overall stud productivity. Vaccine types reported to have a negative effect on sperm quality included PRRS ($n = 7$), SIV [h1n1 or h3n2, or both—in most cases a specific type was not reported] ($n = 8$), PRV ($n = 2$), or a combination of the aforementioned ($n = 2$). A reported 32.7% ± 25.0% of vaccinated boars were affected by vaccination during an event, with a time lapse of 20.3 ± 13.4 days before the negative effects on semen quality were observed. Total recovery of the spermiogram from an event was 5.5 ± 2.7 weeks after vaccination.

Nutritional imbalances can lead to disturbances in boar reproductive function. Excess feed creates overweight boars, which manifest signs of reduced libido. Additionally, overweight boars tend to exhibit an increased incidence of foot and leg problems, leading to their culling. Severely restricted energy and protein in the diet can impair boar reproductive performance. Studies addressing select deficiencies and excesses of vitamins or minerals on boar reproduction are sparse. Selenium deficiency is believed to cause a decrease in sperm production and motility. Zinc deficiency is thought to retard testicular development. Conflicting reports exist on the boar's tolerance to certain mycotoxins (e.g., aflatoxin B_1, vomitoxin, zearalenone) in the diet. As a precaution, routine use of mold inhibitors and binding agents, along with appropriate testing and blending of suspicious grains, can help to minimize their potential effects. Above all, an appropriate and balanced diet is paramount to good boar reproductive performance. Supplementation to a balanced diet should be approached with caution. A typical boar diet can be found in Chapter 98.

Inappropriate management of the boar can lead to subfertility or infertility. It is important that postpubertal immature boars be gradually introduced into service. Maturity of a boar is highly dependent on genotype, and age at maturity can range from 8 to 10 months to well over 1 year of age. Understanding onset of puberty and progression to maturity within genotype is essential to good boar management. Overuse of boars at any age can

result in reduced fertility. General guidelines for boar use can be found in Table 94-1.

Suggested Reading

Althouse GC: Biochemical composition of sperm plasma membranes in normal and heat-stressed boars. PhD dissertation. Iowa State University, Ames, IA, 1992.

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CHAPTER 96

Reproductive Surgery in the Boar

GARY C. ALTHOUSE

Current swine breeding management strategies have placed an increased emphasis on the clinician's technical proficiency in performing boar reproductive surgery. Use of surgically sterilized boars in gilt development and institution of estrus detection programs have become commonplace in many of today's production systems. Critical, scrutinized genetic selection and the preferred distribution of these desired genetic traits through artificial insemination also have placed increased value on the boars that are standing at stud. It is not uncommon for boars selected for artificial insemination programs to have values 25 to 50 or more times greater than their market hog counterparts. These many varied factors contribute to the necessity for a clinician to be able to provide attention to the individual boar's health and reproductive viability and function.

GENERAL ANESTHESIA

Most surgical procedures performed in adult boars require the use of general anesthesia.[1–3] Drug combinations that have been used successfully to provide 30 to 60 minutes of general anesthesia include the following: (1) xylazine hydrochloride (2 mg/kg of body weight) plus tiletamine hydrochloride–zolazepam hydrochloride (Telazol*) (6 mg/kg), administered intramuscularly [IM]; (2) xylazine (1 mg/kg) plus butorphanol tartrate (0.1 mg/kg) plus Telazol (3 mg/kg), administered IM; (3) Telazol (5-ml vial) reconstituted with 2.5 ml of xylazine (100 mg/ml) and 2.5 ml of ketamine (100 mg/ml), with the resulting mixture given IM at a rate of 0.02 to 0.04 ml/kg; or (4)

*Fort Dodge Labs., Fort Dodge, IA.

result in reduced fertility. General guidelines for boar use can be found in Table 94-1.

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*Fort Dodge Labs., Fort Dodge, IA.

premedication with xylazine (2 mg/kg given IM), followed in 10 minutes with intravenous administration of a 5% solution of thiopental sodium, given to effect. Animals should be isolated from herdmates during the recovery period. With any of the surgical techniques outlined, it is preferable to keep recovered boars isolated from other swine for at least 10 to 14 days after surgery to allow healing. Exposed skin sutures should be removed before reintroduction to herdmates, because pigs are curious animals and frequently will pull or bite on the sutures.

REPAIR OF PENILE INJURIES

Penile injuries are not that uncommon in the boar and often are related to facilities and management (e.g., bite wounds in boars kept in group housing or those used in pen and natural mating situations). Primary complaints that could signify a penile injury include the observation of blood in the urine or semen and hemorrhage from the preputial orifice. The challenge to the clinician in addressing penile injuries is the preoperative assessment of the condition to determine if sufficient damage has occurred to warrant medical or surgical intervention. Although penile injuries are not directly life-threatening, if they are not resolved quickly, the boar may become nonfunctional, leading to its early culling.

The penis is best examined while the boar is under general anesthesia. If the penis cannot be easily grasped and exteriorized using gauze pads, use of Bozeman's atraumatic uterine dressing forceps (10.5-inch size) has been found to be helpful for this purpose. If damage to the penis is limited to abrasions or superficial lacerations, adequate sexual rest (for 3 to 4 weeks) is all that usually is required for healing. If, however, urethral patency has been compromised (i.e., fistula formation), especially if located in the body of the penis, surgical correction may be necessary. Trauma to the urethra requiring surgery should be dealt with quickly to avoid the need for excessive débridement. With the animal under general anesthesia, an 18 to 20 Fr Foley catheter is passed into the urethra to act as a stent. After cleansing and débridement, the urethra is closed with a 4-0 absorbable suture, swaged to an atraumatic needle, with a simple interrupted pattern that incorporates the mucosa. Fascia and skin are then closed using 4-0 nylon suture in a simple interrupted pattern. A Malecot drain inserted through a prepubic cystostomy may be used to divert urine from the reconstructed urethral site and thus promote healing; the drain is removed once healing is sufficient for normal micturition. A total of 4 to 5 weeks of sexual rest is recommended before the boar's return to service.

CORRECTION OF PENILE PROLAPSE

Prolapse of the penis and preputial cavity mucosa have been reported after use of neuroleptics (i.e., acetylpromazine) or as a result of trauma to the penis during mounting activity. Penile prolapse should be considered an emergency situation, as additional injury to the penis is likely to occur the longer it remains prolapsed. The length of time elapsed since occurrence of the prolapse and the degree of insult to the prolapsed structures will determine the prognosis after correction.

With the animal under general anesthesia, the prolapsed tissues should be cleansed and examined for extent of injury. To reduce edema, the prolapsed tissues can be massaged during application of a topical antibiotic ointment. If the prolapsed tissues are too edematous to replace, compresses are applied for a short period of time before replacement is attempted. The prolapsed preputial mucosa and penis are then replaced into the sheath by gentle manipulation using the fingers rather than a surgical instrument. Correct repositioning has been accomplished when both the preputial mucosa and the penis are in place behind the ringlike division between the cranial and the caudal preputial cavities. To reduce the chance of postoperative recurrence, a temporary purse-string suture is placed around the preputial orifice. The suture is tightened sufficiently to retain prolapsed tissue but left loose enough to maintain patency for urination. If wounds or abrasions are present, the preputial cavity is flushed with a warm antiseptic solution; systemic administration of antibiotics and anti-inflammatory drugs over a 3- to 5-day period after replacement also is recommended. The purse-string suture is removed after a minimum of 14 days of sexual rest. Sexual rest may need to be extended an additional 15 to 45 days, depending on the severity of the wounds.

CORRECTION OF PERSISTENT PENILE FRENULUM

The frenulum is an epithelial attachment that runs from the mucosal covering of the penis to the base of the corkscrew behind the tip of the penis. This tissue normally is present in prepubertal and peripubertal boars, with breakdown and detachment occurring as a normal event in boars at or just before puberty. With failure of this breakdown process, the penis will "fishhook," or turn backward, when penile extension occurs (Fig. 96-1). Boars with persistent penile frenulum cannot successfully initiate coitus but sometimes can have their semen collected using the gloved hand technique. Depending on severity, surgical correction of this condition can be performed either during semen collection or with the animal under general anesthesia. Resection or, better yet, removal of this tissue is most easily accomplished using scissors. Hemorrhage is minimal, negating the need for ligation before or after excision. The animal can be used for breeding 10 to 14 days after surgery. Because persistent penile frenulum is thought to be heritable, breeding stock should not be selected from the offspring sired by affected boars.

REPAIR OF PREPUTIAL PROLAPSE

Prolapse of the prepuce in the boar is uncommon. In such an occurrence, however, quick action is paramount to successful replacement of viable tissue and minimization of edema. With the animal under general anesthesia, prolapsed tissue should be manually replaced. After replacement, a purse-string suture, using a 1 nonabsorbable monofilament suture, is placed around the preputial

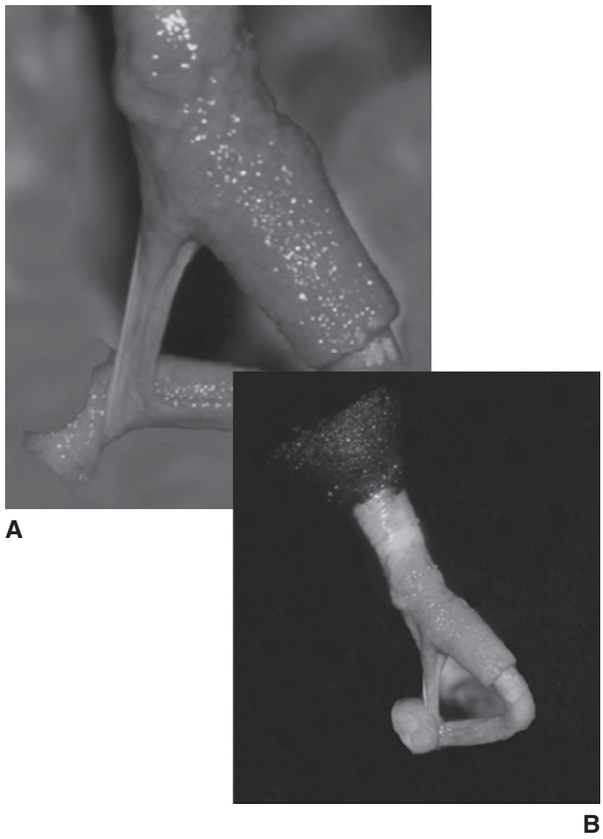

Fig. 96-1 **A,** Persistent penile frenulum in a 10-month-old Hampshire boar. **B,** Magnified view of the frenulum attachment between the internal lamina of the prepuce and the glans penis.

orifice. The purse-string suture should be tightened sufficiently to retain prolapsed tissue yet be loose enough to maintain patency for urination. Flushing of the preputial cavity with a warm, antiseptic solution is often performed daily over a 3- to 5-day period after replacement. At the end of treatment, the purse-string suture is removed, and the boar returned to service after an additional 14 days of sexual rest.

If the boar is presented with a prolapsed prepuce that is edematous and necrotic, surgical correction is warranted. With the animal under general anesthesia, the prolapsed tissue, prepuce, and preputial cavity are surgically prepared. A 1- to 2-cm-diameter tube is then introduced into the preputial cavity to act as a stent. Nonviable tissue is resected, with anastomosis of the two layers of the prepuce using a 1-0 absorbable, monofilament suture in an interrupted pattern. After anastomosis, antibiotic ointment is applied to the area, followed by replacement of the viable tissue back into the sheath. As a precaution, a purse-string suture is placed around the preputial orifice as described earlier. Concurrent removal of the preputial diverticulum should be considered to avoid potential complications associated with prepuce resection or amputation. Postoperatively, 4 to 6 weeks of sexual rest is recommended.

PREPUTIAL DIVERTICULECTOMY

The preputial diverticulum is a bilobed sac located on the dorsal wall of the preputial cavity (see Fig. 94-1). Removal of the preputial diverticulum is warranted to alleviate ulceration and bleeding of the diverticulum; to prevent diverticulitis and abscessation in boars with an enlarged prepuce (i.e., "urine pocket"); to remedy reduced breeding performance due to entrapment of the penis in the diverticulum during attempts at copulation (i.e., "balling up"); and to minimize urine dribbling and odor from the sheath of pot-bellied pigs kept as pets (so-called removal of the scent gland). Preputial diverticulectomy also can be used, albeit uncommonly, to aid in reducing bacterial contamination of semen in boars for use in artificial insemination programs.

Two surgical approaches have been described to excise the preputial diverticulum in swine.[4,5] Both require general anesthesia. In the first approach, the anesthetized animal is placed in either left or right lateral recumbency. The prepuce, including the diverticulum, is flushed with a 2% povidone-iodine solution. While flushing the prepuce and diverticulum, the surgeon can easily visualize the area of the prepuce to prepare for surgery. After surgical preparation of the area, the prepuce and diverticulum are filled and left distended with the disinfectant solution. A skin incision is made on the lateral surface of the prepuce approximately 5 cm caudodorsal to the preputial orifice and is extended caudally along the length and greater curvature of the distended diverticulum (usually 5 to 8 cm). The incision is continued through the subcutaneous tissue and the cranial preputial muscles, with particular caution observed in cutting through the muscles tightly affixed to the diverticulum wall. The thin muscle layer is bluntly dissected away from the fluid-filled diverticulum wall; gauze sponges may be helpful to provide a firm grasp of the thin tissues during dissection. After the most immediate sac has been dissected free, blunt dissection is continued across the top of the diverticulum, directed toward the remaining lateral sac. At the median septum between lobes, the superficial caudal epigastric vessels and supportive connective tissue are encountered. Blunt dissection is performed under these structures and around the remaining sac. Once dissection is complete, the distended diverticulum should be freely movable, with its point of attachment (i.e., neck) to the preputial vestibule. Using a 1 absorbable suture, a purse-string suture is placed around the neck opening but not tightened. The fluid-filled diverticulum is expressed; then, using the fingers, each sac is inverted through the neck of the diverticulum and out the preputial orifice. After sac inversion, the purse-string suture is tightened to reduce the remaining neck opening, and the inverted diverticulum is transected free at the neck. A continuous suture pattern is employed using an absorbable 1-0 or 0-0 suture to eliminate the cavity created by the removal of the diverticulum and to stabilize the cranial prepuce. The skin incision is then closed using a 1 nonabsorbable suture in a Ford interlocking or other supportive pattern. Alternatively, a subcuticular or subcutaneous pattern using an absorbable suture can be used.

A second approach involves using a curved forceps inserted through the preputial orifice to radically dissect the diverticulum and associated tissues, followed by exteriorization of the dissected tissues through the preputial orifice. After complete eversion, an absorbable mattress suture is placed around the diverticulum neck, and the diverticulum is excised. The remaining stump is allowed to retract back through the sheath orifice.

VASECTOMIZATION

Bilateral vasectomy[1,6] commonly is used to sterilize boars. Boars chosen to undergo vasectomy usually are postpubertal, with evidence of good libido. General anesthesia is required. A common surgical approach involves placing the anesthetized boar in dorsal recumbency and securing the legs to allow for easy access to the inguinal area. An area of skin on each side of the sheath extending back toward the scrotum is clipped and prepared for aseptic surgery. A 4- to 6-cm paramedian incision (2 to 4 cm lateral to the penis and 5 to 10 cm cranial to the scrotum) is made caudal to each superficial inguinal ring. Using blunt dissection, the spermatic cord is isolated within the common vaginal tunic and exteriorized (Fig. 96-2). The tunic is incised to expose the closely associated ductus deferens, nerves, vessels, and lymphatics. A 3- to 4-cm segment of the ductus deferens is then ligated using an absorbable suture, and a portion is resected between the ligatures. The common vaginal tunic is then closed using 2-0 absorbable suture, followed by closure of the subcutaneous tissue. The skin is then closed using an absorbable (0) suture in a subcuticular pattern. The same procedure is repeated on the other side.

An alternative approach for vasectomizing boars has been recently developed. The benefit of this approach is that the ductus deferens is easily isolated before incorporation into the spermatic cord, thereby eliminating the chance for damage to associated neurovascular structures that are essential to testicular viability. Additionally, this approach is performed with the boar positioned in lateral recumbency, reducing the anesthetic risk in pigs associated with use of dorsal recumbency. Under general anesthesia, the boar is positioned in either left or right lateral recumbency. The perineal and scrotal regions are surgically prepared. With the testes and scrotum left in their natural position, a longitudinal 2- to 3-cm skin incision is made parallel to the long axis of each testis, starting 1 to 2 cm off the medial septum and 3 to 4 cm medial to the abutment of the cauda epididymidis and capital extremity of the testis and extending caudally (Fig. 96-3). The incision is deepened through the tunica dartos muscle and parietal vaginal tunic, allowing entrance into the vaginal cavity. Entrance into the vaginal cavity is identified by examining the outer surface of the testis, which will have a glistening appearance and prominent vasculature. The ductus deferens, incorporated in the loosely attached mesoductus deferens, is located on the craniomedial surface of the testis. To exteriorize the ductus deferens, a closed Allis forceps is inserted into the

Fig. 96-2 Exteriorized spermatic cord in a dorsally recumbent boar.

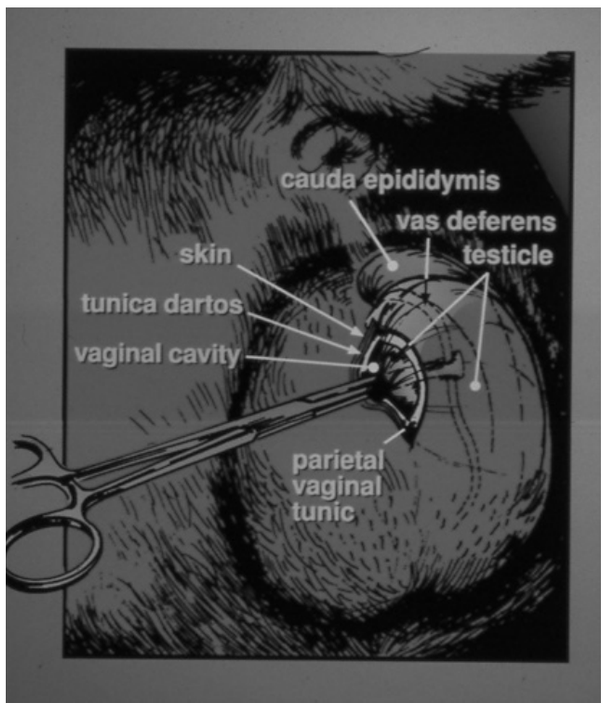

Fig. 96-3 Incision site and position of the ductus deferens in situ for scrotal approach vasectomy. Note the proper placement of the Allis forceps for use in exteriorizing the ductus deferens. (From Althouse GC, Evans LE: Removal of the caudae epididymidis to create infertile boars for use in estrus detection programs. *J Am Vet Med Assoc* 1997;201:676.)

vaginal cavity and directed toward the craniomedial surface of the testis in a slightly open position (Fig. 96-3); then the forceps is closed and the incorporated tissue is exteriorized through the incision site. The ductus deferens is bluntly dissected from the mesoductus deferens. A section of the ductus deferens is then resected between ligatures that have been placed approximately 2 to 3 cm apart. Deeper tissues need not be sutured. The skin incision is closed with 2-0 absorbable suture in a subcuticular or subcutaneous pattern. Alternatively, if the boar will be housed alone, skin closure can be performed using an exposed suture pattern with 0 monofilament, nonabsorbable suture material. The procedure is then repeated on the opposite testis. Antibiotic or antiseptic products, or both, can be applied to the area but are usually not necessary if a clean surgical technique was utilized.

EPIDIDYMECTOMY

Epididymectomy,[7] or surgical removal of the caudae epididymidis, is a simple procedure for creating sterile boars for use in estrus detection programs. This procedure usually is performed on the nonanesthetized neonate pig (1 to 2 weeks of age) in lieu of surgical castration. Chemical restraint may be necessary depending on the boar's size. The neonate pig can be restrained by grasping the hindlimbs, to allow easy access to the scrotum. Owing to the delicacy of the operation, a separate handler often is helpful for physically restraining the boar, allowing the surgeon to have both hands free to perform the operation. The nonpendulous scrotum and perineal area are prepared for surgery using standard techniques. Next, a free finger is used to push on the base of the scrotum, forcing the testes toward the dorsal portion of the scrotum. The cauda epididymidis is located on the dorsal pole (capital extremity) of each testis (Fig. 96-4). A 1- to 2-cm incision is made with a rounded (e.g., No. 20) scalpel blade through the skin directly over the greater curvature of the cauda epididymidis. With constant pressure applied at the base of the scrotum to stabilize the testis and cauda epididymidis, the incision is deepened further, through the tunica dartos muscle and parietal vaginal tunic. The incision through the parietal vaginal tunic should be long enough to allow the cauda epididymidis to be exteriorized but not too wide to allow exteriorization of the testis (Fig. 96-5). Complete exteriorization of the cauda epididymidis is done through the application of pressure at the base of the scrotum or with the use of thumb or Allis tissue forceps. The entire cauda epididymidis is transected free from its ligamentous attachment to the testis and corpus epididymidis using either scissors or a scalpel blade. Care should be taken to ensure that testicular parenchyma is not transected. After the cauda epididymidis is excised, pressure is released from the base of the scrotum, allowing the testicle to return to its normal position in the vaginal cavity. The procedure is repeated on the other testis.

Suturing of the small incisions made through the skin and deeper tissues generally is not required, because the weight of the testes in the scrotum provides for appropriate apposition and subsequent healing of the incision sites. Antibiotics or antiseptic products can be applied to incision sites but usually are not necessary if a clean surgical technique was utilized.

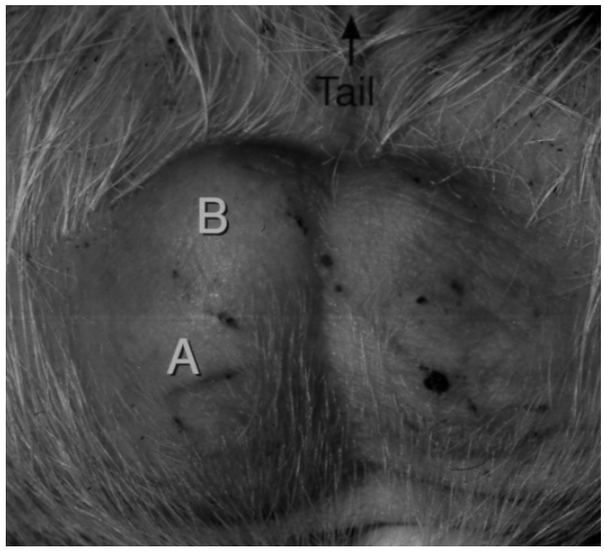

Fig. 96-4 Scrotum of a neonatal boar showing orientation of the left testis **(A)** and the cauda epididymidis **(B).** Notice that pressure applied to the base of the scrotum enhanced the outline of the cauda epididymidis in the dorsal aspect of the scrotum. (From Althouse GC, Evans LE: Removal of the caudae epididymidis to create infertile boars for use in estrus detection programs. *J Am Vet Med Assoc* 1997;210:678.)

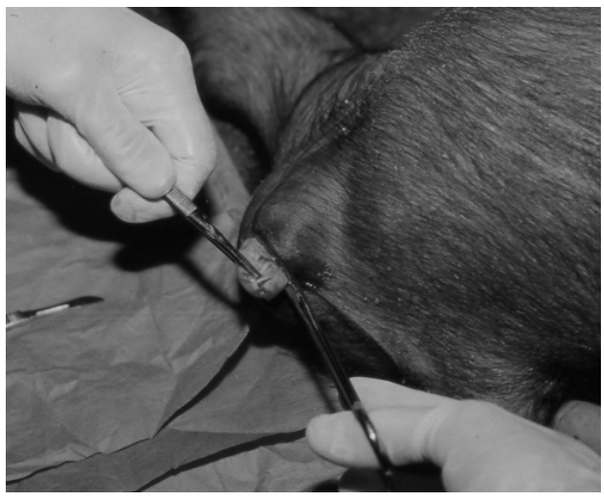

Fig. 96-5 A correctly exteriorized cauda epididymidis ready for transection. The testis is allowed to remain in situ.

CASTRATION

In many parts of the world, bilateral castration is routinely practiced on young pigs destined for market, to curtail "boar taint" of the meat. In commercial production, castration is normally performed by the producer on pigs younger than 2 weeks of age. Using physical

restraint, a longitudinal incision approximately 1 cm long is made through the skin and into the testis using a No. 12 scalpel blade; this incisional depth allows for the testis to "pop out" through the incision. Once exteriorized, the testis is grasped and pulled in such a manner as to separate the cord while minimizing placement of lateral pressure on the inguinal rings.

In certain circumstances, clinicians may be required to perform unilateral or bilateral castration. For larger boars, some form of anesthesia is required. Along with the anesthetic protocols outlined earlier in the chapter, other acceptable anesthetic protocols that have been used in performing castration include lumbosacral epidural block with 2% lidocaine (1 ml/9 kg of body weight) and intratesticular injection of a 30% solution of pentobarbital (1 ml/7 kg of body weight).

The anesthetized boar is placed in lateral recumbency, and the scrotum and perineum are aseptically prepared. An incision (large enough to exteriorize the testis) is made through the scrotum starting at its most ventral aspect, its depth dictated by whether an open or a closed castration technique is performed. For boars with very thick scrotal skin, it may be advantageous to make a stab incision through the scrotum and then lengthen the incision from the inside out. In the preferred closed technique, the testis and vaginal tunic are dissected free and exteriorized. The testis and tunic are then twisted to occlude the spermatic cord up to the level of the external inguinal ring. At this point, either two transfixation ligatures (no. 1 absorbable) are applied to the cord, followed by transection distal to the last ligature, or an emasculator is used to crush and sever the cord at the level of the external inguinal ring.

The open castration technique consists of carrying the incision all the way through and into the testis. The testis and its vascular structures are exteriorized, followed by occlusion of the vascular supply by twisting the cord up to the inguinal ring; either transfixation ligatures and/or an emasculator is used as described earlier with the closed technique. With the open technique, everted tissues (e.g., tunic) must be ablated. For bilateral castration, the medial septum often is excised as well. If asepsis is maintained, the incision site can be closed using an absorbable suture in a subcuticular or subcutaneous continuous pattern. If the boar is housed alone, skin closure can be performed using a monofilament, nonabsorbable suture material in an exposed suture pattern. Suture placement should allow for adequate drainage at the ventral commissure of the incision. In cases in which sepsis is a possibility, the surgical wound can be left open to allow for drainage and left for healing by second intention. Systemic antibiotics should be administered for 3 to 5 days after surgery.

From an economic standpoint, surgical correction of cryptorchidism in the boar is rarely performed. Generally, cryptorchid boars are left completely intact so that they may be later identified at market weight and segregated from penmates at slaughter. Producers are offered less money for intact boars at slaughter, encouraging prudent identification and elimination of breeding stock that carry this undesirable genetic trait.

REPAIR OF TESTICULAR/ SCROTAL INJURIES

Testicular injuries tend to be of traumatic origin and generally are unilateral. Systemically spread infectious agents (e.g., *Brucella suis* or opportunistic pathogens) can elicit an orchitis and/or epididymidis. Testicular and peritesticular injuries constitute an emergency and should be promptly recognized, diagnosed, and treated. Proper diagnosis of the enlargement (e.g., orchitis, periorchitis, hydrocele, hematocele, seroma, hematoma) will aid in determining whether medical or surgical therapy is necessary. Digital palpation along with B-mode ultrasonography has been very helpful for obtaining the information necessary to arrive at a tentative diagnosis. Medical treatment usually consists of aggressive hydrotherapy, anti-inflammatory drugs, diuretics, and, if indicated, antibiotic therapy. If no improvement in the condition is observed over a 5-day course of treatment, surgical drainage or unilateral castration (see earlier) is recommended.

The scrotum has excellent regenerative capabilities. Because of this ability, cleansing the involved area with an antiseptic solution and wet-to-dry dressings usually constitute the only treatment that is necessary. Deep lacerations may benefit from débridement and placement of subcuticular sutures of an absorbable monofilament. If wound sepsis is suspected, open drainage along with systemic or local antibiotics, or both, is indicated.

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CHAPTER 97

Artificial Insemination in Swine: Boar Stud Management

GARY C. ALTHOUSE

DISEASE CONTROL

Control of the introduction of pathogenic organisms into a boar stud operation cannot be overemphasized. Disease control not only is important for overall boar stud health and viability, but also is critical to minimizing the chance of disease transmission to other herds through the extended semen. Disease control consists of the following components: biosecurity, isolation, acclimatization, recovery, and monitoring.

Biosecurity should start with appropriate selection of stud and isolation site locations in order to physically assist in minimizing disease transmission between livestock facilities. Facility environment should be controlled to protect boars from the outside environment. Mandatory restrictions in the movement of animals, personnel, equipment, supplies, and sanitation protocols also are key components in a biosecurity program.

Selection of incoming boar source(s) should be dependent on health status equal to or better than that of the recipient boar stud group. **Isolation** procedures should be utilized on all boars before their introduction to the stud group to protect the recipient stud from the potential introduction of infectious agents that could cause economic distress. Isolation should be off site and away from any other pigs. Animal movement into and out of isolation should be handled in an all in–all out fashion. During isolation, boars should be monitored for signs of, or exposure to, disease through the strategic use of paired serologic tests and daily observation for coughing, sneezing, changes in consistency or amount of feces, decreased appetite or water consumption, skin lesions, lameness, or lethargy.

Acclimatization is used to prepare the replacement boars for the impending challenge of pathogens endemic to the recipient herd and to pathogens being controlled by the stud. Acclimatization starts immediately after isolation and consists of a controlled challenge to these select pathogens to stimulate but not overwhelm the immune system of the isolated boars. Exposure usually is provided through vaccination and direct exposure to young cull boars and their products. Clinical observation and serologic testing should be performed to confirm success of exposure.

The **recovery** phase is provided after acclimatization to allow the isolated boars time to build protective immunity to the controlled, exposed pathogens away from the resident herd. The recovery period also serves to reduce the risk of disease spread to the main stud from new boars still shedding pathogens. The length of time accorded to isolation, acclimatization, and recovery should be dictated by the pathogens that the boar stud operator is trying to control; in general, time periods range from 30 to 90 days.

Once the boar has been moved to the resident stud group, regular **monitoring** of the group should be performed. Monitoring is done through the use of serologic profiling (monthly or quarterly), daily observations of the resident boars, and temporal analysis of semen findings and stud production records.

BOAR HOUSING

Today's boar stud facilities are designed to ensure animal well-being, personnel safety, and ease of movement and handling of boars. Alley width is limited, to allow a boar to freely move in the forward direction but not turn on the handler. To aid in moving the boar into and out of his stall, stalls usually incorporate both vertical entrance and exit gates. Pen partitions are of either solid or open panel design, with vertical bars on open panels to aid in deterring animals from climbing up the panel. For boar movement and cleaning ease, gates, partitions, and panels should be around 1.2 m in height, with a floor clearance of approximately 10 cm. To accommodate variation in boar size (due to their different ages and genetics), stalls of differing sizes usually are included within the same facility.

Nonslip flooring is used in the housing area. Both solid and slotted flooring, either alone or in combination, have been used in boar stud facilities. Solid flooring in the boar housing area requires the use of bedding (e.g., straw, wood shavings) to keep the area and animal dry. Slotted flooring, the most common type of flooring in modern swine facilities, incorporates space between cement slats that allows for the free passage of waste into a pit. Passage of urine and feces into a pit minimizes soiling of the animal and eliminates the need for bedding. From a hygienic standpoint, total slotted floors are preferable to either solid or a combined slotted/solid floor for housing boars.

Semen collection should be performed in a pen specifically designed for this task. Because personnel are operating close to the boar, the pen should be designed to allow multiple escape sites out of the pen if the boar becomes aggressive. Two or three of the perimeter walls should be constructed using a 5-cm-outer-diameter (OD)

Fig. 97-1 Porcine semen collection area and dummy.

galvanized pipe. This pipe should be approximately 1 m in height and placed at 0.3-m intervals on center. The height and the space (approximately 0.25 m) between pipes should allow personnel to easily enter and exit the collection area without having to open or close gates but restrict the boar to the pen area. Collection pens should be 1.8 to 2.4 m in width and 2.4 to 2.75 m in length. A stable collection dummy (Fig. 97-1) should be placed in the collection pen. Added features of collection dummies may include capability for height adjustment and some type of support on the sides of the dummy that allow the boar to stabilize his front legs during mounting and thrusting. It is paramount that a nonslip surface be provided around the dummy so that the boar has secure footing during mounting and thrusting. It may be helpful to position the collection dummy in a corner of the pen or with one end against the wall so that the boar can easily be directed and maintained around the dummy. The pen and the collection dummy should be situated away from boars and other distractions that would divert the boar's attention away from the collection dummy. Some stud operators design their facilities to include a pen—commonly called a "warm-up" pen—that is in close proximity to the semen collection pen. This pen is used to queue a boar so that he observes and hears the actions of another boar in the collection pen; these stimuli frequently help to excite the boar, so that when he is moved into the semen collection pen, reaction time to mount of the dummy sow is minimal, and semen collection can be accomplished more efficiently.

BOAR NUTRITION

Traditionally, nutritional needs for boars were thought to be similar to those for gestating sows. The goal of these traditional programs was to provide boars with adequate caloric intake to meet their maintenance requirements, while minimizing weight gain. Under natural mating conditions, minimizing the weight gain of boars was critical to their length of usefulness in the herd, because gilts and sows have finite physical limitations on the amount of weight they can bear by the boar during the mating process. Once boars became too heavy, they were culled. This simple approach to defining boar feeding programs is still applicable for boars used under natural mating conditions. In today's global swine industry, however, artificial insemination (AI) has taken over in many breeding programs. With this change, the traditional feeding strategy does not appear to meet the nutritional needs of an AI boar, for which productivity is measured through sperm production (i.e., number of extended semen doses/ejaculate) rather than servicing capabilities.

Much early work addressed the effects of restricted nutrient intake on boar reproductive performance. Restrictions or deficiencies in protein,[1,2] energy,[2] and certain vitamins and minerals[3,4] have been shown to negatively affect boar reproductive performance. Conversely, overfeeding and excess body conditioning also can have a negative impact on boar reproduction. Ideally, adequate nutrition should be provided to active boars to meet their requirements for maintenance, growth, breeding activity, and sperm production. In general, feed intake is adequate in young (<16 months) boars if they show moderate (400 g/day) weight gains, and in mature boars if they show slight (200 g/day) weight gains. In practice, body condition scoring most often is used as an indicator of health and adequate feed intake. Depending on genetics and structural conformation, a body condition score of 3 to 3.5 (on a scale of 1 to 5) is a desirable target. To obtain a more objective assessment, backfat monitoring by means of B-mode ultrasonography also is being used to monitor boar body condition.

Feed constituents for boar diets vary widely and usually are dictated by local availability. Cost has become less important than with other swine diets because the boar ration is fed to only a small but valuable number of animals in a herd. A typical ration used in an AI boar stud operation contains approximately 3000 metabolizable energy (ME) kcal/kg, 16% to 18% crude protein (0.85% lysine), 4% fat, and 5% fiber.[5] Generally, a boar will consume 1.5 to 3.0 kg of this ration on a daily basis. Daily allowances may be modified according to season and environmental conditions. Thermoneutrality for the boar is approximately 20° C.[6] If temperatures fall below 20° C, the amount of feed provided to boars should be increased by 0.08 kg/day for every 1° C decrease. During periods of warmer ambient temperatures, it is not advisable to decrease feed amount, because this may lead to an unbalanced diet. Instead, energy density of the diet may be reduced to decrease body heat production but still maintain the ration's balance. Nutritional supplements such as certain vitamins, minerals, and fatty acids have been advocated as aids for improving or maintaining boar semen quality and quantity under stressful environmental or nutritional conditions; further research is warranted.

On some farms it is not feasible to have a separate diet and storage area for the small number of AI boars housed on the property. To address this problem, supplements that contain high levels of certain nutrients are available that can be top-dressed onto feed used in gestation or lactation diets.

BOAR TRAINING

When the boar is introduced to the semen collection area, sufficient time should be allowed for the animal to investigate and become comfortable with the surroundings. Patience is required in training boars for semen collection using a mounting dummy. Prior exposure of naive boars to the semen collection process by allowing them to observe, in an adjacent pen, a boar having semen collected often can be helpful. After the boar identifies the stationary dummy, some animals will readily mount it and begin to thrust, whereas others may need to be gently directed toward the dummy. Once the boar shows interest in the dummy, he is quietly approached from the rear or side (behind the shoulder). During this time, sudden movements, loud noises, and moving into and out of the boar's visual field should be avoided, to minimize the chance of startling the boar. To stimulate thrusting and exteriorization of the penis, the assistant gently reaches around and massages the boar's prepuce. Once the penis is exteriorized, digital pressure is applied to the spiral-shaped glans penis. If properly stimulated, the boar will fully extend his penis. Once it is extended, thrusting will cease and ejaculation will commence.

Training periods should be limited to no more than a single 15-minute interval once daily. Daily training periods should be provided until semen collection is successful. After a successful event, reinforcement training of the semen collection process should be provided on at least 2 consecutive days before resting the boar or placement into a regular collection schedule.

SEMEN COLLECTION

Contamination should be minimized when semen is being collected for use in an AI breeding program[7] (Box 97-1). Application of digital pressure to the penis using the gloved hand technique is the most popular method for collecting boar semen. Nonspermicidal gloves (e.g., vinyl, nitrile) should be used by the semen collector; some glove materials (e.g., latex) have been found to be spermicidal in this species.[8] The semen collector should place two gloves on the collecting hand. After the boar has mounted the collection dummy and has commenced thrusting, the collector reaches underneath the boar and massages the sheath. Sheath massage will allow for evacuation of contaminant preputial fluids and aid in exteriorizing the penis. After the penis is exteriorized, the top glove is discarded. Holding the semen collection container (e.g., a prewarmed, insulated thermos or Styrofoam cup with a filter over the opening) in the free, ungloved hand, the collector then kneels down and grasps the boar's corkscrew-shaped glans penis with the gloved hand. Constant pressure is applied to the glans penis with the fingers, so that the glans penis is "locked" in the hand; this grasp mimics the cervical lock that occurs during natural mating. Pressure applied elsewhere on the penis may elicit a negative response. If adequate pressure is applied, the boar will respond positively by fully extending his penis.

Ejaculation begins with emission of the presperm fraction (i.e., the first few jets of an ejaculate); this fraction is not collected. Sperm-rich and sperm-poor fractions are next emitted and the fraction(s) collected. Gel components, originating from the bulbourethral gland, normally are emitted in conjunction with the sperm-poor fraction. The gel fraction is of little value when semen is to be used in an AI program and is therefore separated from the fluid portion of the ejaculate by filter or gauze over the opening of the semen collection vessel. Once the boar has completed ejaculation, the raw ejaculate is protected from chemical (e.g., water, soap residues, alcohol), thermal (hot or cold), and light (i.e., ultraviolet) insults.

Box **97-1**

Techniques for Minimizing Contamination in Swine Semen Collection/Processing for Artificial Insemination

Boar Preparation/Semen Collection

1. Trimming of hair around the preputial opening
2. Use of double gloves, with the outer glove discarded after preparation of the boar, allowing for a clean gloved hand for grasping the penis
3. Using disposable vinyl gloves or hand disinfectant to reduce risk of cross-contamination between boars
4. Cleansing preputial opening and surrounding area (if needed) with a single-use disposable wipe
5. Manually evacuating preputial fluids before penis is grasped for semen collection
6. Holding penis perpendicular to the boar to minimize contamination of semen collection vessel with preputial fluid
7. Diverting initial jets of ejaculate (i.e., presperm fraction) and final gel fraction from the semen collection vessel
8. Disposing of rubber band and filter/gauze before the collected semen is sent to the laboratory for further processing

Semen Processing/Laboratory Sanitation

1. Using disposable products when economically feasible
2. Cleaning reusable laboratory materials (i.e., glassware, plasticware, plastic tubing, containers, and so on) that cannot be heat/gas sterilized or boiled, with a laboratory-grade detergent (residue-free), followed by multiple rinsings in distilled water and then in 70% alcohol (nondenatured), with sufficient ventilation for complete evaporation of residual alcohol
3. Rinsing reusables with semen extender before their first use of the day
4. Disinfecting countertops and equipment at the end of each processing day with a residue-free detergent
5. Mopping the floor at the end of the day with a disinfectant (e.g., phenolic, formalin product)
6. Breaking down bulk products into smaller, daily-use quantities
7. Installing ultraviolet lighting to aid in sanitizing reusables and laboratory surfaces
8. Using proper safety precautions to protect personnel from exposure

Data from Althouse GC, Kuster CE, Clark SG, et al: Field investigations of bacterial contaminants and their effects on extended porcine semen. *Theriogenology* 2000;53:1167–1176.

It is important to maintain the boar on a routine semen collection schedule. Both overusage and underusage of an AI boar may have a negative impact on herd fertility. Suggested guidelines for boar usage are presented in Table 94-1.

SEMEN EVALUATION

From a physiologic standpoint, several attributes appear to be necessary for a spermatozoon to fertilize an oocyte (Box 97-2). Because these necessary attributes are multiple and complex, it is easy to understand that a test of any single one of these attributes would, and does, correlate poorly with overall fertility. Ideally, determination of semen quality would consist of a test that examines all of these attributes. Unfortunately, a practical and simple in vitro test that can be routinely performed before ejaculate processing to determine the quality (e.g., fertility potential) of an ejaculate has yet to be developed. Several in vitro tests are available, however, that can examine selected attributes of sperm necessary for natural fertilization. Although individually these tests have limited usefulness in determining fertility potential, collectively they do have the ability of screening out overtly poor-quality semen.

Good-quality boar semen is essential to satisfactory herd fecundity rates. Accordingly, most progressive boar stud operations routinely assess every ejaculate collected at their facilities. Standard tests in evaluating boar semen include the cursory examination of color and odor, with more detailed analysis of sperm motility, sperm morphology, sperm concentration, volume, and total sperm numbers. Suggested indicators of quality for freshly collected boar semen to be processed and used for AI are indicated in Table 97-1.

After collection, the ejaculate should be visually assessed for color. A normal ejaculate will be gray-white to white in color and have a milky opacity. If the ejaculate exhibits abnormal color (e.g., brown, yellow, red) or a strong urine odor, contamination should be suspected. Since many of these contaminants exhibit spermicidal activity, these ejaculates should be discarded.

Sperm motility should be evaluated. The percentage of motile spermatozoa usually is determined using microscopic analysis. Accuracy of this subjective assessment is highly dependent on sample preparation, microscope quality, technician experience, and natural ability. To minimize the effects of human error, sample preparation and microscopic assessment should be standardized. The most common technique is to place a 7- to 10-µl sample of semen on a warmed (37° C) microscope slide, overlaid with a coverslip. When viewed under a microscope, the sample should appear as a monolayer of cells within which individual sperm motility is easily visualized. In some instances, neat semen may be too highly concentrated to be assessed without first diluting the sample with an equal-sized drop of a isothermal diluent before overlaying with a coverslip. Sperm motility is estimated to the nearest 5% by viewing sperm activity in at least four different fields on the slide at 200× or 400× magnification and then taking the average of these readings to obtain the final motility estimate. Sperm motility and viability normally decrease during storage; therefore, ejaculates should exhibit at least 70% to 80% gross motility to be considered for further processing.

Once sperm motility is determined, morphology should be evaluated. In the swine industry, both wet-mount and dry-mount preparations are used for objective quantitation of sperm morphologic abnormalities. With wet-mount preparations, sperm should be immobilized using a buffered formalin or glutaraldehyde solution. Wet-mount slides are then examined with microscopes that provide their own internal contrast (e.g., phase or differential interference contrast). A variety of contrast stains are used for morphologic study of boar sperm using dry-mounted slides. Proper contrast stains accentuate the outline of the sperm, allowing for easier visualization and identification of normal and abnormal sperm under the light microscope. To make a stained slide, equal volumes (e.g., 7–10 µl) of stain and sample are applied adjacently to a microscope slide. The drops are gently mixed together using the edge of a second slide. The edge of the second slide is then used to draw the mixture across the slide to produce a thin layer, which is allow to air dry. Under oil immersion, sperm morphology is assessed. For both wet- and dry-mount techniques, a minimum of 100 sperm should be morphologically assessed and categorized. Normal boar ejaculates generally exhibit less than 15% to 20% abnormal sperm.[9,10]

Box **97-2**

Sperm Attributes Necessary for Oocyte Fertilization

- Progressive motility
- Acceptable morphology
- Ability to undergo capacitation (i.e., plasma membrane modifications)
- Ability to undergo acrosome reaction (i.e., vesiculation/exocytosis)
- Ability to penetrate cumulus investments
- Ability to bind and penetrate the zona pellucida
- Sperm-oocyte plasma membrane fusion

Table **97-1**

Minimum Requirements for Use of Fresh Boar Semen for Artificial Insemination

Semen Variable	Descriptor/Value
Appearance	Milky to creamy consistency
Color	Gray-white to white
Gross motility (unextended)	≥70 % (if used ≤ 48 hr) ≥80% (if used ≥72 hr)
Abnormal morphology	≤25%*
Cytoplasmic droplets†	≤15%

*The 25% maximum includes cytoplasmic droplets.
†Includes proximal and distal cytoplasmic droplets.
Data from Althouse GC: *Boar stud management.* CAB International, 2002.

The next step in ejaculate evaluation is determination of sperm concentration. The most common method of determining sperm concentration in gel-free boar semen is by measuring the degree of sample opacity using a photometer. In boar semen, opacity is dependent on the number of sperm cells and other seminal plasma components that interfere with the transmission of light through the sample. Boar semen normally is too opaque for light to pass readily through it; therefore, a small sample of boar semen is diluted into an isotonic solution before obtaining a measurement. For photometric measurement to be relatively accurate, it is essential that the instrument calibration be species specific. Additionally, owing to inherent differences between photometers, conversion charts should not be interchanged between instruments. Inaccuracies in photometric measurements can occur if readings fall outside the optimal operating range of the equipment or may be due to human error (e.g., incorrect dilutions, improper warm-up time, solution mishandling, delayed reading); moreover, innate differences commonly are found between boar ejaculates. Manufacturer recommendations should be followed.

Sperm concentration in boar semen also can be determined using a dilution apparatus and a counting chamber, although this is uncommon. A portion of the neat semen is first diluted 1:200 using a sperm-immobilizing, isotonic solution. This mixture is then charged onto each chamber of the hemacytometer. After 5 minutes, to allow sperm to settle onto the grid surface, the hemacytometer is placed under a microscope at 200× to 400× magnification for sperm counting. A minimum of 5 large (80 small) squares are counted in the center (RBC) grid on each side of the hemacytometer. Only sperm heads (i.e., not tails) touching the top and left lines of the large square are included in the count; those touching the bottom or right lines are not counted. The two counts are then averaged if they are within 10% of each other. If the two counts vary by more than 10%, the counts are thrown out, and the hemacytometer is again prepared and the two side counts repeated until the counts are within 10% of each other. This number *(N)* is then inserted into the formula supplied by the distributor of the hemacytometer at a 1:200 dilution to determine the number of sperm cells per milliliter of semen. The time required for hemacytometric counts, combined with their tediousness, makes them impractical for most AI laboratories. Photometric analysis remains the most commonly used technique for determining sperm concentration per milliliter of gel-free ejaculate.

Sperm concentration is next used to determine total sperm numbers in the ejaculate. Total sperm numbers are calculated by multiplying the total gel-free ejaculate volume with the sperm concentration. Ejaculate volume usually is determined by weighing the ejaculate, with the assumption that 1 g is equal to 1 ml.

Computer-automated semen analysis systems specific for the swine industry have been recently introduced. In addition to performing sperm motility and concentration assessment, these systems also come with software to assess sperm morphology. With technician input, these computerized systems have the ability to grade an ejaculate, calculate total sperm numbers, and determine the amount of diluent to add to the ejaculate to achieve the desired sperm per dose. Because of their speed of semen assessment and increased accuracy over existing methods, several large, high-volume stud operations have implemented use of this equipment.

Several other tests have been promoted for assessing boar semen quality and include various sperm-binding assays, hypo-osmotic swelling test, osmotic resistance test, selected biochemical assays of sperm and seminal plasma, and fluorophore assays. These tests can assess factors associated with sperm or the ejaculate other than those gained through routine assessment of sperm motility, morphology, and concentration. Because of the time, tedium, or expense involved in performing these tests, their routine use in boar stud operations has been limited to date.

SEMEN PROCESSING

Porcine semen extenders are readily available from many commercial vendors. Depending on the extender chosen, extended boar semen can be stored for up to 6 days with no appreciable loss in fertility potential.[11] Most recently, semen extenders purported to maintain sperm viability for up to 10 days are being marketed; field fertility trials, however, are equivocal at this time. In general, most commercial farms use extended boar semen within 3 to 4 days of processing. A majority of semen extenders come in powdered form and are reconstituted using purified (NCCLS/CAP Reagent Grade Type I or II) water. Reconstituted semen extender should be incubated at 37°C for 45 to 60 minutes to allow pH equilibration before use with semen.

Fully diluted porcine semen should have a final sperm concentration of 25×10^6 to 80×10^6 sperm cells/ml. Final sperm concentration is determined on the basis of the expected duration of storage of the product before use. As a general rule, if the ejaculate meets the minimum criteria for neat boar semen to be used for AI, then 1 billion spermatozoa per dose is included for every day of storage (e.g., if the extended sample will be stored for 2 days, 2 billion sperm/dose is used; if for 3 days, 3 billion sperm/dose; if for 4 days, 4 billion sperm/dose; and so on.). Depending on extender capacity, dilution rates of 1:4 to 1:25 (ratio of semen to extender) are used. If sperm concentration cannot be determined for the ejaculate, a conservative dilution of 1 part ejaculate to 4 to 7 parts diluent should be employed, with the extended product used within 24 hours of dilution. To maintain viability of a fresh ejaculate during processing, isothermal extender should be used during the dilution process.

After the ejaculate has been processed with appropriate extender, individual insemination doses are produced. Three different receptacles currently are used to store extended porcine semen doses: bottles, tubes, and bags/flatpacks (Fig. 97-2). These receptacles are made of nonleaching plastic materials and are nontoxic to spermatozoa. These receptacles are constructed to be flexible, rather than rigid, so that the receptacle collapses naturally with the female's uptake of the semen. Most currently marketed receptacles are designed to hold an insemination dose of up to 100 to 125 ml. Current dose

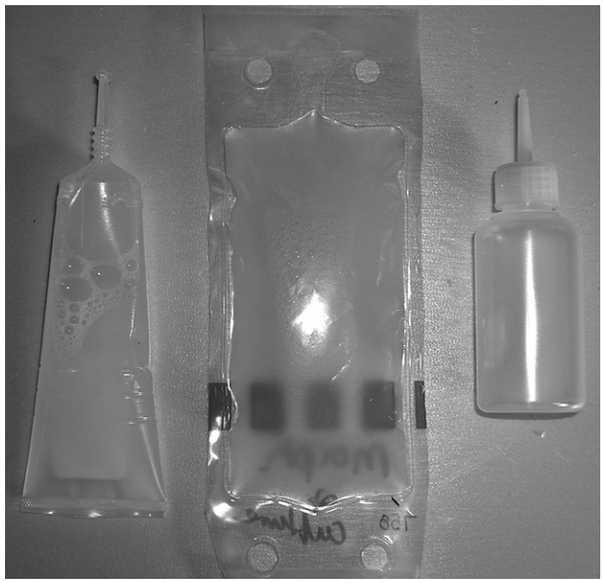

Fig. 97-2 Tube, flatpack, and bottle storage receptacles commonly used for holding extended porcine semen doses.

volumes used by the industry for cervical insemination are 70 to 85 ml. Final sperm concentrations, sperm/dose, and dose volumes may be reduced in the future if transcervical insemination is employed.

Mixing or pooling semen from different boars has become a common technique for processing boar semen for AI. Pooled semen has been found to yield good fertility results in swine.[12–21] The benefits of pooling semen are twofold. First, pooling semen tends to increase processing efficiency in the laboratory by allowing for a large number of boar ejaculates to be processed simultaneously. Second, pooling semen provides the producer with a means to reduce, or even eliminate, inherent differences in fertility found between sperm and between boars. Pooling semen is a simple process. Freshly collected ejaculates are examined for semen quality, as discussed previously, to eliminate any overtly poor-quality ejaculates. Processing of ejaculates for pooled semen can be performed in any of several ways. In one common method, good-quality ejaculates are diluted 1:1 with an extender and placed in a water bath (20° to 37°C, depending on laboratory processing protocols, type of extender, efficiency of laboratory, and so on) to maintain constant temperature while collecting/analyzing/diluting subsequent ejaculates that will be added to the semen pool. Another method of pooling involves adding ejaculates to a fixed amount of extender (e.g., 500 ml) until the target number of doses is reached. With either method, the number of boar ejaculates to be pooled together should not exceed the volume capacity that the operator can process at one time. A minimum of 3 ejaculates per pool is recommended. After the final ejaculate is added to the pool, the pooled sample is diluted to its final calculated volume with the remaining extender. The pooled sample is then further processed, handled, and stored using standard protocols. For large volumes, it may be necessary to provide constant mixing of the sample during packaging to prevent sperm settling, which can lead to variation in sperm concentration between doses.[22]

ADDITION OF COMPOUNDS TO SEMEN

A variety of hormonal compounds (e.g., estrogens, oxytocin, prostaglandins) have been added to semen before insemination in an attempt to increase overall fecundity. Compounds tested have been selected on the basis of their known actions in physiologic events that are important to successful reproduction. To date, their addition to semen has yielded equivocal results. The physiologic basis for the variation observed in response to these compounds is not known and requires further study.

SEMEN STORAGE

Storage temperatures range from 15° to 18°C for extended boar semen. Exposure of extended porcine semen to higher temperatures can increase utilization of the extender components, may induce terminal biochemical modifications to the sperm membrane, and, logically, may enhance bacterial growth of contaminated samples. Conversely, temperatures at or below 10°C may cause irreversible damage to both sperm motility and acrosome integrity.[23] Depending on extender type, stored semen should be gently agitated at least twice a day to resuspend sperm cells within the extender dose.

FROZEN SEMEN

A uniformly successful freezing and thawing protocol for porcine spermatozoa still eludes the industry. Certain boars and breeds appear to exhibit better sperm freezability than others, confounding results. Owing to the reduced longevity of frozen-thawed porcine semen in the female reproductive tract, insemination timing in relation to ovulation also is a contributor to its reproductive performance. Overall, reduced farrowing rates and litter sizes result from the use of frozen-thawed porcine semen in breeding programs. In light of this expected decrease in fecundity, frozen semen has established its value to the swine industry through its long-term preservation of valuable genetics, convenience for over broad distances and international lines, and availability for emergency use by farms when access to fresh extended semen is interrupted.

A variety of freezing methods have been used, including pellets, small straws (e.g., 0.5 ml), large straws (3.0, 5.0 ml), and flatpacks.[24] In swine, breeding doses frequently contain large sperm numbers (e.g., 6 to 8 billion), necessitating the handling of moderate-sized dose volumes. To handle this size breeding dose, freezing methods employing large straws and flatpacks are desirable. Current practices call for transferring frozen-thawed boar semen into a bottle containing diluent. This diluted, frozen-thawed dose is then inseminated by cervical deposition, as with fresh extended semen. Recently, the industry has been experimenting with use of a new type of transcervical disposable catheter (Fig. 97-3). If this method proves to be superior in ease of deposition of

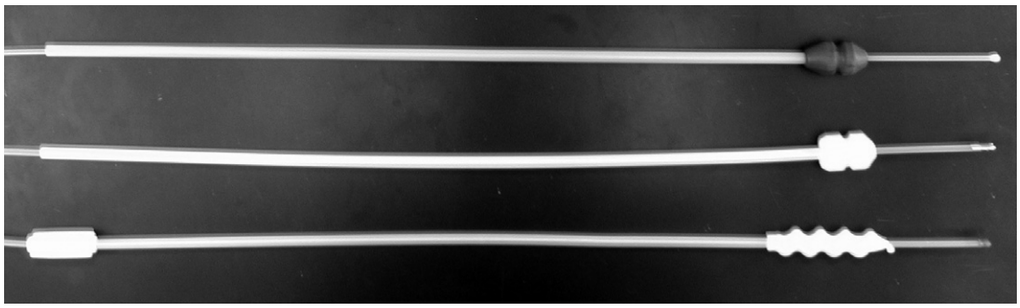

Fig. 97-3 Porcine transcervical artificial insemination catheter.

Box **97-3**

Ranked Reasons* for Boar Turnover in U.S. Boar Stud Operations

1. Poor semen quality
2. Normal genetic turnover
3. Structural problems
4. Behavioral problems
5. Death
6. Disease

*Most common to least common.
Data from Althouse GC, Kuster CE: A survey of current boar stud practices in USA production. In Cargill C, McOrist S (eds): Proceedings of the 16th Congress of the International Pig Veterinary Society. Melbourne, Australia, 17–20 September 2000. Rundle Mall, South Australia: Causal Productions, 2000, p 398.

semen into the uterine body or horn, transcervical AI may facilitate use of frozen-thawed boar semen.

BOAR CULLING

Boars generally enter isolation and then the resident stud group between 6 and 8 months of age. In the United States, culling rates approach 15% during the isolation period alone.[25] Reasons for culling during quarantine, in order of frequency from most to least, were (1) poor conformation/lameness, (2) poor libido, (3) poor semen quality, (4) death, (5) serologic or clinical confirmation of presence of an unwanted disease, and (6) overly aggressive behavior toward handlers.

In resident stud groups, annual boar culling rates average 59.6%.[25] The average breeding life of a stud boar is 20 months. The most common reasons by frequency for boar culling are presented in Box 97-3. Culling of boars due to poor semen quality occurs at 58.2 days if by this time no improvement is seen in the spermiogram.

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CHAPTER 98

Clinical Reproductive Physiology and Endocrinology of Sows: Mating Management

TIMOTHY J. SAFRANSKI and NANCY M. COX

GENETIC SYSTEM

The swine industry has embraced crossbreeding systems for several years. The two factors making crossbreeding systems superior are breed complementation and heterosis. **Breed complementation** is simply using breeds or lines in a manner to take maximum advantage of their genetic type. Each breed or line has evolved in response to selection for specific characteristics. Terminal breeds and lines have been selected primarily for growth and body composition (e.g., Duroc, Hampshire, Pietrain), whereas emphasis has been placed on reproductive performance in maternal breeds and lines (e.g., Yorkshire, Large White, Landrace). Crossbreeding systems are designed to maximize use of terminal lines as boars and of maternal lines as sows, as, for example, with a Duroc boar mated to a Yorkshire sow.

Heterosis, or hybrid vigor, is the other factor leading to adoption of crossbreeding systems. **Heterosis** is simply the tendency for crossbred progeny to perform differently from the average of their parents. In the Duroc × Yorkshire example, the average expected daily gain of Durocs is 0.88 kg and that of Yorkshires is 0.84 kg. The crossbred progeny would be expected to gain 0.86 kg daily without heterosis. Heterosis for daily gain typically is approximately 9%, so progeny would actually be expected to gain 9% better than the parental mean, or 0.94 kg daily.

Heterosis tends to be highest for traits with the lowest heritability, those most susceptible to environmental variation. Reproductive and fitness performance, therefore, benefits most from heterosis. This includes embryo survival, litter size born, neonatal survival, and so on. For traits such as neonatal survival, a crossbred pig benefits from individual heterosis, and if the dam is crossbred and produces more milk, the piglet also benefits from maternal heterosis. A summary of heterosis values for some economically important traits is presented in Table 98-1.

Heterosis may be thought of as recovery from mild inbreeding associated with development of breeds or lines. Accordingly, it is not surprising that heterosis is beneficial to the pig for all traits, and to the producer for most traits, backfat being the exception. In rare instances, heterosis may not justify crossbreeding. If only two lines of pigs were available for use in a maternal line, one with a litter size of 8 and the other with 10 pigs, an F_1 hybrid, with heterosis, would be expected to have (8 + 10) ÷ 2 + 10% heterosis = 9.9 pigs. Although this litter size is better

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Table **98-1**

Individual and Maternal Heterosis Estimates for Selected Traits

	INDIVIDUAL HETEROSIS		MATERNAL HETEROSIS	
Trait	**Absolute**	**Percent**	**Absolute**	**Percent**
Ovulation rate, number	0.04	0.3		
Conception rate, %	3.00	3.8		
Litter size born	0.52	5.1	0.71	6.8
Litter size at 21 days	0.56	8.0	0.64	8.7
Postweaning average daily gain, kg	0.06	9.4	0.0	0.0
Postweaning gain/feed ratio	0.02	2.3	0.0	0.0
Carcass backfat, cm	0.08	2.5	0.13	4.4
Longissimus area, cm^2	0.52	1.78	0.12	0.4

Data from Johnson RK: *Heterosis and breed effects in swine.* North Central Regional Publication No. 262. Washington, DC: ARS, USDA, 1980.

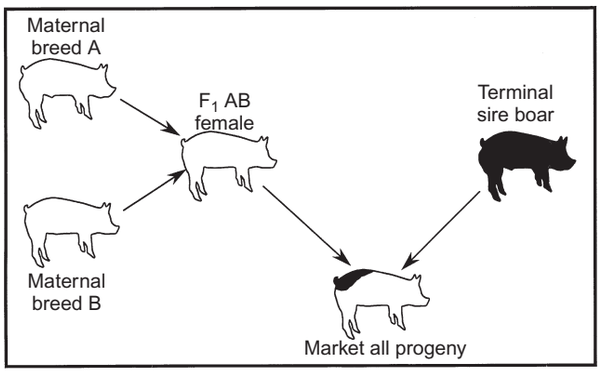

Fig. 98-1 An example of a terminal crossbreeding program.

than the parental mean, it is inferior to that of the better parent.

Figure 98-1 shows a crossbreeding system that maximizes heterosis and breed complementation. For this system to work, the F_1 AB female will need to be either produced on farm or purchased. Systems purchasing the F_1 AB maternal line female are referred to as parent systems. Those purchasing the pure breed A and breed B to produce F_1 AB females are referred to as grandparent systems. Great-grandparent systems are similar but use an F_1 female instead of the A or B breed. On-farm production of replacement females typically requires a commitment of 10% to 20% of the sows for replacement gilt production. Semen can be purchased and artificial insemination (AI) used to eliminate the need to maintain all of the boar lines.

The advantage of grandparent systems over parent systems is the reduced number of incoming animals. The disadvantage is that the progeny must be managed by the farm until ready for mating. This results in the need to raise maternal line pigs with market hogs. Fine-tuning management can be complicated in this scenario because the maternal line pigs typically grow differently and have different nutritional demands. Maternal line barrows may not fit marketing specification in some cases.

REPRODUCTIVE ANATOMY

Understanding the basic reproductive anatomy of the female pig is critical to trouble-shooting reproductive problems, at both the individual and the herd level. Reproductive organs, feet and leg soundness, and the suitability of other body systems to support long-term reproductive performance should be evaluated in potential replacement females.

Structural Soundness

Feet and legs of potential breeding animals should be free of injuries. Hoof cracks, sole bruises, foot rot, subsolar abscesses, and obvious injuries of the upper legs are reasons to cull a potential breeding animal. Uninjured and normal-appearing feet and legs should be examined for general structural soundness. Legs that are too straight or have too much slope to the pastern area may predispose the animal to leg injuries or leg weakness.

External Genitalia

Normal vulval size varies; however, once gilts begin cycling, the vulva should enlarge noticeably in comparison with that of the prepubertal animal. Infantilism, or extremely small size of the vulva, is a common abnormality of the reproductive tract and usually is associated with a small uterus and ovaries and failure to cycle.

A dorsally tipped vulva occasionally is observed. Although not known to be associated with problems with the internal genitalia, in a natural mating system the physical structure of the dorsally tipped vulva makes intromission difficult for some boars.

Male pseudohermaphroditism can occur in pigs. Animals with this condition usually have female external genitalia but also have testes, or they may have gonadal tissue of both sexes. The testes may be located subcutaneously within the scrotal area but often are located

within the abdomen. Male pseudohermaphrodites often have a "fishhook" or "sky-tipped" vulva.

Internal Genitalia

Although not available for examination except at slaughter or by real-time ultrasound evaluation, the internal genitalia of gilts may be affected with abnormalities that prevent normal reproductive performance. Hydrosalpinx and pyosalpinx are conditions that result from obstruction or distention of the oviducts, allowing them to fill with fluid, thereby blocking the passage of spermatozoa and ova. These conditions possibly are the result of abnormal embryonic development and may be hereditary. If both oviducts are affected, the gilt will be infertile but may have regular estrous cycles. Females affected in only one oviduct are prone to repeat breeding and reduced litter size.

Segmental aplasia in the body of one or both horns of the uterus may result in complete or partial infertility. Affected animals have normal reproductive cycles, with pregnancy possibly occurring in nonaffected segments of the uterus, but subsequent litter size is small.

A blind or missing cervix occasionally may be noted. Affected gilts usually cycle but do not become pregnant. Adhesions in the oviduct and around the ovaries are relatively common in sows and gilts. Normal cyclicity is possible, but litter size is usually reduced if pregnancy does occur.

Mammary System

The anatomy of the mammary system should be examined in all potential replacement animals. The underline should be well developed, with a minimum of six functional teats on each side, with three in front of the umbilicus. Nipple abnormalities such as blind teats (not fully developed), pin nipples, or inverted nipples are reasons for rejection of gilts as breeding animals. Sows should maintain a minimum of 10 functional teats throughout their productive lives. Abscesses and injuries may lead to nonfunctional teats, so regular examination is necessary.

ESTROUS CYCLES

Endocrinology

Changes in reproductive hormones during the estrous cycle have been well characterized[2–4] (Fig. 98-2). The length of the estrous cycle in swine is approximately 21 days. For the purposes of this discussion, the cycle is divided into the follicular phase (proestrus), the ovulatory phase (estrus), and the luteal phase (diestrus). Patterns of circulating hormones and intraovarian events are described.

The **follicular phase** is the period from luteal regression to ovulation. Luteal regression, as indicated by a decline in circulating progesterone concentrations, occurs between 13 and 15 days after the previous estrus. This decline in progesterone is caused by the uterine luteolysin, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), in the utero-ovarian circulation. During this time, follicles destined to ovulate enter a period of accelerated growth resulting from increased frequency of episodic luteinizing hormone (LH) release after the progesterone decline. Increases in LH receptors on ovarian graafian follicles promote increased synthesis and secretion of follicular estradiol. Estradiol concentrations peak in serum between days 18 and 20 of the estrous cycle, due to increased follicular aromatase enzyme activity. Approximately 40% of follicles present on days 15 to 16 of the cycle undergo atresia or degeneration before the LH surge (day 20 or 21) (Fig. 98-3). Follicles that go on to ovulate reach diameters of 5 to 12 mm before ovulation.

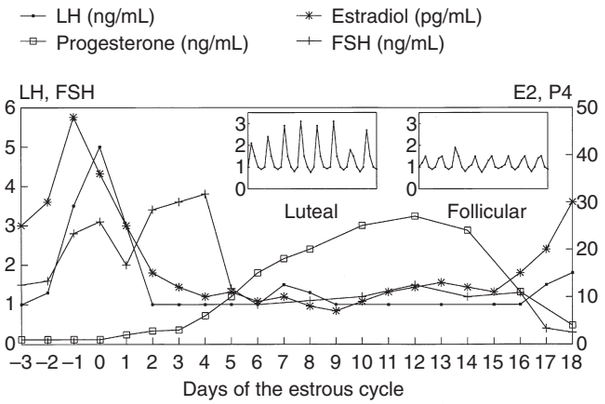

Fig. 98-2 Average concentrations of reproductive hormones during the estrous cycle in swine. *Insets* represent luteinizing hormone concentrations in samples collected every 10 minutes for 7 hours during the luteal and the follicular phases, respectively. (Data from references 1 to 3.)

The rise in estradiol during the follicular phase is responsible for triggering the preovulatory surge in LH and producing estrous behavior. Sexual receptivity lasts 1 to 4 days, with older animals in estrus longer. In most animals, ovulation occurs 36 to 44 hours after onset of estrus. Some of this variability in duration of the **ovulatory phase** can be explained by duration of estrus. Regardless of length of standing heat, sows ovulate two thirds to three fourths of the way through estrus. A sow in heat for 24 hours would be expected to ovulate 16 to 18 hours after onset of estrus. A sow that stands for 72 hours would be expected to ovulate 48 to 54 hours after onset of estrus. Natural ovulation in sows, determined using transrectal real-time ultrasound evaluation, lasts about 2 hours.[5] Other studies have determined the duration to be up to 9 hours. Duration may be affected by technique and by mating, which advances ovulation.[6]

The LH surge reduces estradiol production and shifts steroidogenesis to progesterone production. The oocyte resumes meiosis in response to the gonadotropin surge, and meiosis is completed after sperm penetration.

The role of follicle-stimulating hormone (FSH) during the ovulatory phase has been less well characterized than that of LH. A surge in FSH coincident with the LH surge has been reported, but the postovulatory surge in FSH is

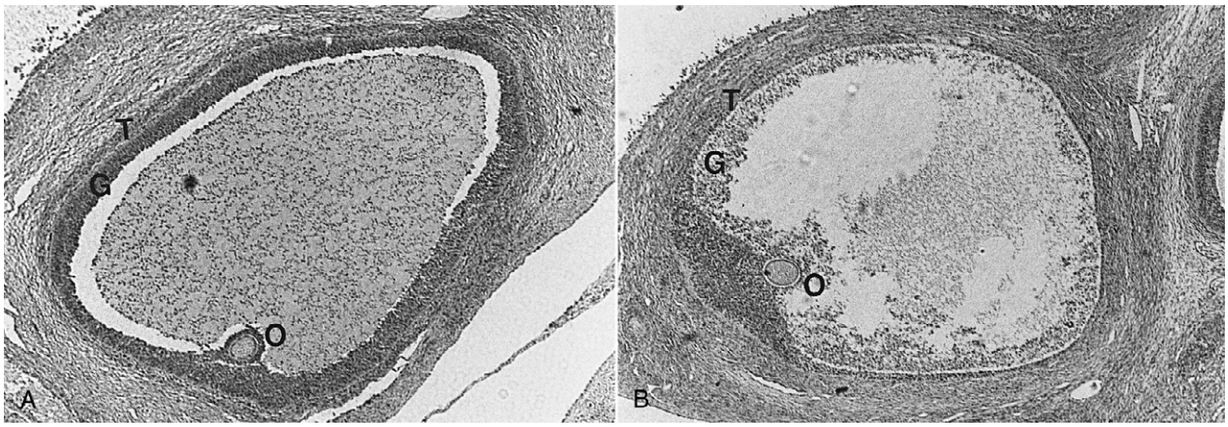

Fig. 98-3 Light micrographs of ovarian follicles (hematoxylin and eosin, original magnification 40×). In the nonatretic follicle **(A),** an intact granulosa layer (G) surrounding the ovum (O) and an intact theca layer (T) are visible. In the atretic follicle **(B),** the granulosa layer is fragmented owing to extensive degeneration of granulosa cells.

more distinct and appears to be responsible for activating small follicles for potential ovulatory growth during later cycles. Circulating FSH is inversely related to concentrations of inhibin, a follicular protein known to exert negative feedback on FSH. Inhibin is produced increasingly as follicles reach ovulatory size.

The **luteal phase** is the period of corpora lutea formation and progesterone production. Increasing progesterone is evident in serum between 2 and 4 days after estrus. Corpora lutea are formed from the cellular remnants of follicles after ovulation, which transitionally are called corpora hemorrhagica. As with other species, the corpora lutea of pigs contain identifiable cells of follicular granulosa (large luteal cells) and thecal (small luteal cells) origin.

Other hormones that have been characterized during the estrous cycle include prolactin, which increases at estrus and appears to have a luteotropic role during the estrous cycle and pregnancy. Cortisol also increases at the time of estrus and when prepubertal females are exposed to a boar as part of the puberty induction process.

Ovulation Rate

The number of ova released at estrus (ovulation rate) depends on the number of preovulatory follicles that develop and their rate of atresia. Ovulation rate is affected by age, breed, and nutrition. Ovulation rate increases by a factor of about 2 from pubertal to third estrus, although the way in which gilts are fed also influences ovulation rate. In full-fed gilts compared with gilts on restricted feed, ovulation rates are elevated. Flush feeding restricted-fed gilts eliminates the depression associated with limit feeding. **Flush feeding** refers to the practice of elevating feed intake for several days to 2 weeks before mating. The increase in ovulation rate is not always accompanied by increased litter size, presumably as a result of increased embryonic mortality. In instances in which increased litter size was observed, feeding excess energy was terminated at the time of estrus and did not continue through early pregnancy. Few studies, however, have systematically described specific uterine effects of elevated dietary energy.

Increasing ovulation rate has been reported with the use of immunization against inhibin and androstenedione, as well as with treatment with epostane, an inhibitor of progesterone synthesis, and gonadotropin-releasing hormone (GnRH). Metabolic manipulation with insulin and growth hormone also has been suggested to increase ovulation rate. Currently no therapies are approved for increasing ovulation rates in swine, so genetic selection, crossbreeding, and nutritional manipulation are the only avenues available.

PREGNANCY

Embryo Survival and Development

It generally is agreed that for sows when successful fertilization occurs, nearly 100% success in fertilization is achieved in swine. Between 20% and 30% of potential embryos (as reflected by number of corpora lutea) die during the first 30 days of gestation, and another 10% die before term. It appears that in most instances, embryo death occurs between days 13 and 20.[7] Some proportion of embryo deaths is due to chromosomal aberrations and congenital lethal defects that are unavoidable. Porcine embryos migrate into the uterine lumen from the oviduct at about day 4, and growth is supported by uterine histotrophs. Embryos begin to secrete estradiol at approximately days 10 and 11 of embryonic life, coincident with rapid development from the spherical to the filamentous stage (Fig. 98-4). A second increase in estradiol occurs after day 14. The estradiol secretion is associated with specific uterine protein secretion, uterine epithelial cell morphology and secretory patterns, maternal recognition of pregnancy, and embryonic migration.[7] Embryo size at this transitional stage of development can vary. Recent information indicates that strains of pigs with greater litter size, such as miniature swine selected

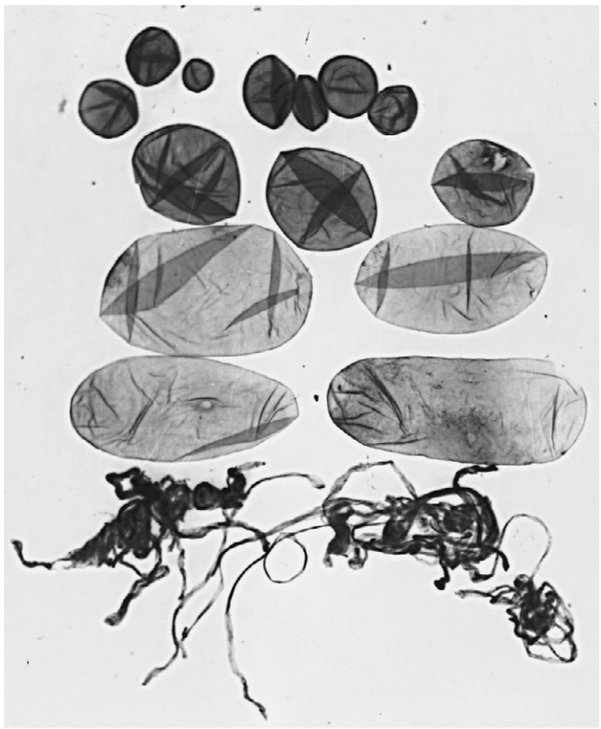

Fig. 98-4 Early porcine conceptuses collected between days 10 and 12 of gestation. Sizes range from 3-mm spherical blastocysts *(top row)* to tubular (15 mm length) to filamentous blastocysts. (Courtesy of Dr. Rodney D. Geisert, Oklahoma State University.)

for the D haplotype and Chinese Meishan swine, have blastocysts that grow more slowly and secrete estradiol more gradually than do domestic swine. As a result, intrauterine competition for space may be reduced during the critical period of distribution and elongation of embryos. Another hypothesis for variation in embryo development is that the later ovulations give rise to smaller embryos, and that initiation of estradiol production by older blastocysts inhibits this maturational process in the younger embryos. This hypothesis was not supported by research results showing that embryonic diversity at 98 hours was not related to duration of ovulation.[5]

Few therapies are available that reliably increase embryonic survival. Maintaining sows in a cool, calm environment for several hours after breeding seems to increase embryo survival. Limiting feed intake to a maintenance level for 2 to 5 days after breeding also has been suggested to be beneficial to embryo survival, presumably because of the relationship of intake with circulating progesterone levels.[8] This effect is seen more often in gilts than in sows. Parity 1 and sometimes parity 2 sows typically have a reduced subsequent litter size. A practice of mating these females at their second postweaning estrus rather than the first has been shown to increase litter size dramatically, and embryo survival has been proposed as the mechanism. A high level of hygiene and ensuring that matings occur only during sexual receptivity will enhance efficiency of reproduction and could be partly mediated through embryo survival.

Uterine Capacity

Although ovulation rate places an absolute limit on litter size, embryo and fetal survival depend on the complex uterine factors described. A practical term for these collective uterine influences is **uterine capacity.** Several investigations have proved the importance of uterine capacity when ovulation rate is not limiting. For example, the ovulation rate and number of fetuses are correlated linearly up to a certain point, but then the magnitude of this association levels off, being described by most researchers as a quadratic relationship. When ovulation rate is greater than uterine capacity, increased embryo or fetal death (calculated as a percentage of the ovulations) occurs. An increase of 3.7 eggs was realized from nine generations of selection for ovulation rate, but only 20% of the increase was reflected in an increase in litter size.[9] Direct selection for litter size for eight generations in the high ovulating line resulted in an increase of 1.06 in number of piglets, presumably as a result of enhanced uterine capacity. Uterine capacity may involve several mechanisms including physical size, degree of folding between uterine and placental tissues, and vascularity of the placenta.

POSTPARTUM PERIOD

Endocrinology

During lactation, the ovary is relatively inactive, with few follicles developing to larger than 5 mm in diameter. LH is suppressed during lactation, whereas FSH is not suppressed as severely, but ovarian factors (presumably inhibin) effect some suppression of FSH. On weaning of the litter, an increase in pulsatile secretion of LH occurs. The increased LH, together with the FSH present, stimulates follicular development, which culminates in a preovulatory LH surge and ovulation. In normal animals, this results in estrus about 3 to 7 days after weaning.

The mechanisms causing some sows to fail to come into estrus after weaning are not well understood. In many sows, an increase in estrogen occurs but induction of an LH surge does not. Later on, the hypothalamopituitary mechanisms appear to recover from the inability to produce a surge in LH, but this period is variable, and anestrus can persist for more than 1 to 2 months. Wean-to-estrus interval (WEI) is discussed later in the chapter.

Prolactin is elevated during lactation and returns to baseline after weaning. This hormone is not thought to be involved in delaying returns to estrus after weaning, because estrus can be reliably induced in the face of elevated prolactin levels. Oxytocin also is increased during suckling in lactating sows and is increased during feeding but does not appear directly involved in postweaning anestrus.

Metabolic factors are thought to affect return to estrus, but inconclusive results are obtained when feeding level between weaning and estrus, or body condition at

weaning, is examined. Static measures of metabolic status appear less sensitive than dynamic measures. It is known that sows will sustain milk production by catabolizing body fat and protein in the face of limited intake. The level of digestible lysine intake required to prevent protein catabolism resulting in reduced subsequent litter size was established as 45 to 48 g per day for first-parity sows.[10] In another study in which feed intake was experimentally limited, WEI was extended in limit-fed sows, but no additional benefit was achieved with hyperalimentation.[8] No differences were observed in ovulation rate or embryo survival, although numbers of sows per treatment were not large. Factors affecting metabolism in gestation clearly interact with metabolic factors during lactation and after weaning.

PREPUBERTAL PERIOD

Limited ovarian follicular growth occurs until the age of approximately 60 days in swine. Primordial and primary ovarian follicles are observed at approximately 68 and 75 days of gestational age in the fetus, respectively, and secondary follicles are observed at the time of birth. Tertiary follicles, recognized as gonadotropin-sensitive, appear between 60 and 90 days of postnatal life.[1] Concentrations of gonadotropins are elevated during the last few weeks of the fetal period and 3 to 4 weeks after birth; then LH is suppressed and FSH is fairly constant until puberty[11–14] (Fig. 98-5).

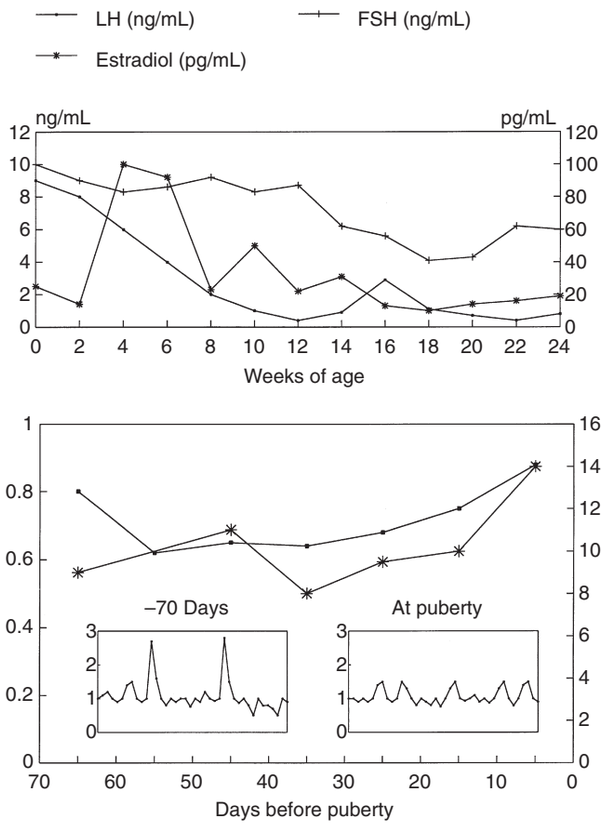

Fig. 98-5 Average concentrations of reproductive hormones of gilts according to age *(top panel)* and days from onset of puberty *(bottom panel)*. *Insets* in the bottom panel represent luteinizing hormone concentration in samples collected every 10 minutes for 7 hours at 70 days before puberty and at the time of puberty. (Data from references 11 to 13.)

PUBERTY

Endocrinology

Estrogen concentrations are elevated during the fetal period but decrease soon after birth. The first sustained rise occurs at the time of puberty, coincident with the first development of follicles to preovulatory size (5 to 12 mm).

The age of puberty ranges from 5 to 8 months, depending on genetic and environmental factors including season, social environment, and nutrition. For most common maternal lines under routine management, gilts are capable of expressing estrus around 165 days of age. Gilts not in estrus by a farm-specific maximum age should be culled, because they are likely to experience inferior reproduction throughout their lives. It is generally agreed that the final stage of preovulatory ovarian follicular development at puberty is due to an increase in LH secretion. Patterns of LH are lowered before puberty, presumably because of increased steroid negative feedback. The gradual increase in LH generally is taken to support the gonadostat theory, which describes the phenomenon of reduced hypothalamic responsiveness to negative feedback of ovarian steroids as puberty approaches. Several studies have demonstrated that LH pulse amplitude is reduced as gilts near puberty, but one study that measured LH in frequent samples up to the time of puberty found increased LH frequency immediately before puberty (see Fig. 98-5).

Responsiveness of the ovary to gonadotropins and the pituitary-ovarian axis to GnRH has been used to infer developmental aspects of puberty. The ovary responds to exogenous gonadotropins with follicular growth and ovulation as early as 100 days of age, although this response improves as gilts approach puberty.[14] Gilts 70 days of age responded to GnRH with no ovulation or a low ovulation rate. Administration of GnRH to gilts 100 days of age or older produced typical cyclic patterns of estradiol and LH and supported normal ovulation rates. It is clear that the ovary and the pituitary are competent in advance of puberty and await maturation of hypothalamic signals.

Gilt Development

Variation in productivity among sows can be attributed to several factors. Gilts will routinely have lower productivity than multiparous sows. The degree of this difference depends on gilt development and sow management programs. A discussion of events associated with puberty is appropriate at this point.

Once a gilt is identified as a potential replacement female, she should be managed as such, with provision of the best environment possible. If cross-fostering is

practiced, potential replacement gilts should be preferentially fostered to smaller litters. From weaning through early grow-finish, they can be managed with conventional production technologies. Historically a common practice was to pull gilts from the finishing phase immediately before mating. With changes in marketing age, space allowance, and feeding protocols, this practice cannot be endorsed.

Nutrition

Feeding replacement gilts conventional finishing diets *ad libitum* until market weight is an acceptable practice. Although the scientific literature concurs that full feeding results in the youngest age at puberty, with advanced growth rates observed in modern production, it is common to limit feed for replacement gilts for some period before mating. Limited research data support limit feeding of gilts to increase sow longevity. To achieve the benefits of limit feeding without subsequent negative production effects, it may be useful to flush-feed as previously described. Manipulation of diets is necessary depending on whether gilts are being fed *ad libitum* or with restricted diets, and use of a gestation diet is recommended when limit feeding is used. The National Research Council (NRC) provides several tables and a computer model to predict daily nutrient requirements for pigs of various stages.[15] For a gestating female expected to consume 1.96 kg of feed daily and gain 55 kg during gestation, a diet containing 3400 kcal/kg DE and 12.9% crude protein, 0.75% calcium, and 0.60% phosphorus is recommended. The female should then consume 11.4 g of total lysine daily.

Housing/Temperature/Photoperiod/Air Quality

Older literature suggested that growth performance would suffer from overcrowding before reproductive performance. Some systems today provide floor space known to limit growth of individual pigs because it results in greater gain for the total building. Accordingly, replacement gilts should be identified early and provided with a minimum of 0.74 square meter until 114 kg of liveweight is attained. As gilts enter the breeding herd, space allocation goes up dramatically. Recommendations are presented in Table 98-2.[16]

Gilts housed in very small groups (less than three) or very large groups (greater than 30) exhibit delayed puberty. Use of heat checks in groups larger than 12 typically is associated with reduced efficiency of estrus detection, so pens of 6 to 12 gilts should be considered ideal.

Other housing factors important for gilt development include temperature, photoperiod, and air quality. In practice, these factors often are confounded. Constant elevated ambient temperatures have been demonstrated to delay puberty. Poor air quality likewise delays puberty, independent of the effects on growth. From experimental data it is not possible to detect how much of this delay is due to a physiologic delay and how much could be due to interference with the ability to respond to olfactory cues associated with boar exposure. Data on photoperiod are more equivocal. Minimum or maximum photoperiods have been suggested, but results from controlled studies are not consistent. If a constant photoperiod is to be provided, a day length between 10 and 16 hours probably is the safest. Results of an experiment help to demonstrate part of the reason for disagreement among studies. The wild pig is known to be a seasonal breeder. The investigators found if day length was extended to 15 hours during periods of decreasing day length it helped to reduce the age at puberty. During periods of increasing day length, however, no benefit was observed.[17]

Comparison of housing systems is more difficult. Most scientific data suggest that outdoor production is superior to confinement with regard to gilt development. In these studies, however, differences in the various components previously discussed usually existed: space allocation, social environment, photoperiod, air quality, and so forth.

Boar Exposure

Many operations develop sophisticated gilt development programs but fail to effectively incorporate the most consistent and dramatic factor: boar exposure. The "boar effect" has been described and studied in detail.

It is commonly believed that boars reach sexual maturity at 7 to 9 months of age. If sexual maturity is defined as the ability to breed sows or gilts, this is largely accurate. Further maturation, however, occurs in the broader sexual system of the boar. Specifically, although sperm production and serum testosterone reach nearly mature levels by the age of 7 to 9 months, the submaxillary salivary gland does not mature until 10 to 11 months.

Table **98-2**

Recommended Minimum Space Allowances for Sows and Gilts

	GROUP PENS		INDIVIDUAL STALL	
Production Group	**Size (kg)**	**Solid Floor (m^2/head)**	**Slatted Floor (m^2/head)**	**Slatted Floor Dimensions (cm)**
Breeding gilts	114–136	3.72	2.23	61 × 213 × 102
Breeding sows	136–227	4.46	2.79	61 × 213 × 102
Gestating gilts	114–136	1.86	1.30	56 × 213 × 107
Gestating sows	136–227	2.23	1.49	61 × 213 × 107

Adapted from MWPS-43, *Swine breeding and gestation facilities handbook.*

It is in these glands that circulating testosterone is converted to pheromones, specifically, 5α-androstenone. The pheromones have two effects on females. In prepubertal gilts, the pheromones are the most significant component of boar effect that can stimulate puberty; this effect is referred to as a **primer effect.** Additionally, they can elicit lordosis or standing heat, an effect referred to as a **signaler effect.** It is critical that consideration be given to which effect is being sought in a particular situation.

To maximize the effect of the boar at stimulating puberty, a minimum of 10 minutes daily of full physical contact will yield the maximum effect comparable to that observed with 24-hour contact. Housing gilts with mature boars for prolonged periods increases their risk for injury, so 10 to 15 minutes is preferred. A compromise that requires less labor is to provide fenceline exposure continuously. This will capture 50% to 90% of the primer boar effect. If fenceline contact is used, the housing should provide as much nose-to-nose contact as possible, because olfactory cues are the most important of the various boar stimuli.

Miscellaneous

Besides the boar effect several factors are claimed to stimulate puberty in gilts. Among the most commonly mentioned are transportation, relocation, and mixing in a new social environment. Transportation alone has been shown to be ineffective. The relocation and mixing often associated with transportation, however, can help to induce puberty. Capitalizing on this phenomenon is not always a simple task. Historically, gilts could be moved to the breeding unit from finishing areas at appropriate ages—160 to 165 days for most lines. Although that still appears to be an appropriate age, the scientific literature lacks data regarding the optimal procedures given the additional moves associated with isolation and acclimation on many farms today.

Genetic variation exists among lines and breeds for several gilt development traits. An extreme example is the very early age at puberty for Chinese Meishan pigs of 90 to 120 days, versus 140 to 180 days for conventional breeds. Variation in other production traits, including longevity, lifetime productivity, WEI, gestation length, and milk production, also is likely. Unfortunately, limited data exist with current genetic lines/breeds. Fortunately, most reproductive traits are associated with a high level of heterosis. By using crossbred females, therefore, reproductive performance can be enhanced.

Manipulation of Estrous Cycles

Manipulation of estrous cycles is discussed in depth in Chapter 100. Induction of puberty through pharmacologic procedures may be a viable management tool. The role of FSH and LH in growth and ovulation of follicles has been described previously. For gilts physically capable of cycling but still prepubertal, the natural system can be mimicked with FSH and LH. As an alternative, pregnant mare's serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG), compounds with FSH- and LH-like activities, can be used. A commercially available combination of PMSG and hCG, PG-600,* is approved for induction of puberty. Reported results have been good.[18] Producers often fail to achieve as good a response. Reasons are not known, but several factors can contribute to variation in response. Best results are obtained when this agent is used in conjunction with other stimuli (moving and mixing, boar exposure). It is critical that the label dose be administered intramuscularly. A common problem observed in the field is administration at an inappropriate age. Gilts that are too young are unable to respond or unable to maintain normal cycles, as previously described. This treatment also is ineffective in gilts that are already cycling. In practice, a proportion of gilts often are cycling before the initiation of estrus detection, and often at significantly younger ages than has been realized. If this problem is suspected, a slaughter check or real-time ultrasound evaluation of ovaries of presumed prepubertal gilts can be useful. Alternatively, blood samples can be collected for progesterone analysis. PG-600 often is used at or near weaning at times when poor return to estrus is expected (e.g., late summer).

In cycling females the only effective methods to synchronize estrus involve manipulation of the corpus luteum (CL). Pregnancy results in maintenance of the CL and cessation of cyclicity, and lactation results in suppression of cycles, as previously described. Unlike in cattle, swine CL are not responsive to $PGF_{2\alpha}$ until late in the estrous cycle (days 12 to 14). This makes administration of prostaglandin to cycling gilts an impractical method to control estrous cycles. Two alternatives exist. One is to prolong the life of the CL by mating the gilt. In gilts that conceive, the CL will persist, and remain responsive to prostaglandins for several weeks. Treatment with $PGF_{2\alpha}$ between days 14 and 35 of pregnancy typically induces regression of the CL, abortion, and a return to estrus in 4 to 5 days. Not all gilts respond, however, and provision must be made for those maintaining pregnancy. This procedure works only for those gilts conceiving, so a high conception rate is critical. Although demonstrated to be effective as a research tool, this protocol does not have label approval.

Another effective method involves use of a synthetic progestin, allyltrenbolone (Regumate*). Extensive research has been done with this product demonstrating that it can be fed at 15 to 18 mg/day to prevent estrus. Gilts and sows typically show normal estrus and ovulation 4 to 5 days after feeding of the product has ceased. Although used extensively in many parts of the world, this product is approved for use only in mares in the United States and is considered too costly to be used as a routine management tool in swine production. Another mechanism to deliver progesterone has been through the use of intravaginal devices for controlled internal drug release (CIDRs). Results are not consistent, with the development of follicular cysts occurring in some groups. CIDRs also are not currently available for use in swine.

*Intervet, Millsboro, Del.

Wean-to-Estrus Interval

Variability in WEI is attributable to many factors, some relatively responsive to management. First-parity sows will have a longer WEI than that observed in multiparous sows. This probably is unavoidable, and management systems should be designed to accommodate this fact, especially in start-up or expansion periods when the herd contains a disproportionate number of parity 1 females. WEI tends to be shortest with lactation lengths near 28 days. After parturition, several physiologic and physical changes must occur in the sow before successful subsequent reproduction, as previously described. The uterus must undergo some degree of involution, a process that is not complete for some 24 days in lactating sows. Sows not allowed to nurse at all have been found not to begin cycling for at least 8 to 10 days after parturition.

Seasonal delays in WEI are to be expected in late summer and early fall. This is part of what is commonly referred to as seasonal infertility, and the causes are not completely clear. Minimizing heat stress, ensuring fresh feed, and providing cool water can reduce this effect.

Litter size weaned has been repeatedly demonstrated to affect WEI. Nursing more and larger pigs places greater metabolic demand on the sows. Management to prevent these sows from reaching a catabolic state becomes increasingly important.

Field reports of delayed WEI in sows weaning very small litters also exist. Although this has not been investigated in controlled studies, the longer WEI in these sows is likely to be from a different reason. With very small litters, six or piglets or fewer, for example, suckling-induced inhibition is not adequate to prevent return to estrus during lactation. A proportion of these sows will return to estrus in the farrowing crate and not be observed. At weaning, therefore, rather than being acyclic, they are in the luteal phase of the cycle and will not exhibit estrus until regression of the CL.

Lactation feed intake is perhaps the single most important factor affecting WEI. During lactation a sow that does not consume enough nutrients will use body stores to support milk production. A reduction in body fat often is expected during lactation, but significant loin muscle loss also can occur and is associated with reduced subsequent reproduction.[10] Nutrient density should be adjusted for realized lactation feed intake. Conditions vary among farms, but to maximize feed intake some elements are constant. A comfortable environment for the sow should be provided for as much of the day as possible; lights should be on when temperatures are in the thermoneutral zone; presence of fresh feed should be ensured. Multiple daily feedings almost always increase feed intake. A regimen of at least twice-daily is suggested. Feeding more frequently may be beneficial if periods between feedings are of sufficient duration. Providing wet feed will enhance feed intake for some sows but has no effect on others. Regardless of whether feed is wet or dry, the feed in front of sows must be fresh. This will typically require discarding leftover feed once daily. Low lactation feed intake usually is indicative of an environmental factor that will respond to management.

The final element affecting WEI is exposure to a boar after weaning. Most farms begin boar exposure on the first day of estrus detection, typically day 3 or 4 after weaning. This practice evolved because detection of estrus is the primary objective. Limited data suggest that boar exposure at weaning can shorten the WEI. With this practice, consideration must be given to the difference between stimulation of estrus and detection of estrus.

Factors favoring shorter WEI also tend to favor other reproductive parameters. In retrospective analyses, sows with shorter WEI tend to have increased farrowing rates and subsequent increased litter size. This advantage is not related to length of WEI per se but derives from the fact that WEI is serving as an index for reproductive fitness. The implication is that systems must be designed to optimize reproductive performance, and WEI is a simple measure of this complex trait.

Mating Systems

An understanding of the physiology of the sow and gilt helps in the design and optimization of mating systems. Successful mating systems require that adequate supplies of viable semen be present in the oviduct at the appropriate time relative to ovulation. The use of real-time ultrasonography has allowed observation of ovaries of nonanesthetized sows at regular intervals and accurate description of time of ovulation relative to onset of estrus. In conjunction with timed inseminations, this knowledge has confirmed data regarding success of matings.

Insemination not more than 24 hours before or 4 hours after ovulation results in near maximum farrowing rate and subsequent litter size. Sperm cells must undergo capacitation in the female tract before fertilization, and this takes about 4 hours. After capacitation, a population of sperm cells exists in the oviduct that are slowly released from oviductal crypts over several hours. After 24 hours the number or quality of sperm cells released declines, with a consequent decline in fertility. Once ovulated, eggs remain viable for a relatively short time, 4 to 8 hours. A bioeconomic model was developed using these data to compare the performance of various mating schedules using once- or twice-daily detection of estrus.[19] The stochastic model demonstrated that the optimal timing of inseminations depends on frequency of estrus detection. Generally the model supports the practice of multiple inseminations, but a 12-hour interval rather than a 24-hour interval between inseminations is suggested. Conclusions might differ if intervals were not 12 hours, and further evaluation of the model under field conditions is needed.

The quality of the mating also is important. Although limited research has been conducted, field data suggest that having a boar present at insemination may increase farrowing rates by as much as 10%. Olfactory cues seem to be the most critical element, so fenceline exposure is adequate. Some farms find success by dangling rags soaked with boar saliva in front of sows during insemination. What role the boar's presence plays is not entirely clear. Certainly it will help to induce lordosis, which is associated with uterine contractions. Perhaps the greatest

effect is that it confirms that the sow is still in estrus. This helps the breeder to be calm. Historically, several investigators have demonstrated the improved fertility with multiple inseminations. A negative impact of a third insemination has been demonstrated when that insemination was given in late estrus or after estrus.[20]

The challenge to timing of inseminations is to predict time of ovulation. Ovulation seems to occur at two thirds to three fourths of the way through estrus, as previously discussed. This finding provides little value as a management tool because by the time prediction of ovulation becomes possible, it has already occurred. Understanding the relationship of WEI and duration of estrus, however, has led to the development of specific mating protocols based on WEI. Sows with short WEI tend to have longer estrus than sows with longer WEI. For example, sows returning to heat at 4 to 5 days after weaning might have first mating delayed for 24 hours, sows returning after 6 to 7 days might have a 12-hour delay, and sows returning after 7 days might be mated at first detection of estrus. Obviously this depends on the duration of estrus expected on a given farm.

Estrus Detection

Pen mating systems rely on the boar for detection of estrus. All other mating systems are predicated on the accurate detection of onset of estrus by breeding personnel. Lordosis, or standing heat, usually is identified by signs such as swelling and reddening of the vulva, vocalization, boar-seeking behavior, "ear popping," and standing for back pressure. Lordosis involves chronic flexing of the muscles by the gilt. This posture cannot be maintained indefinitely owing to muscle exhaustion. When a gilt or sow is in estrus but unable to display lordosis, she is said to be refractory. Because of this phenomenon, it is important that boar exposure be delayed until the time of estrus detection. Ideally, the boar is housed separately from the females. Gilts and boars can be moved to a neutral area for estrus detection, or the gilts should be moved to the boar area. All gilts exposed to the boar must be observed simultaneously, so groups of 6 to 12 gilts are ideal. If it is not possible to house boars and gilts separately, they should be housed as far apart as possible. General agreement is lacking on the separation distance required. Although a 76-cm aisle may represent an adequate distance in some cases, a greater distance often will be required. It also is important to address direction of air currents. In a tunnel-ventilated barn, for example, boars should be kept closer to the exhaust than gilts that will be observed for estrus. Because of the unpredictable nature of air currents in naturally ventilated facilities, a greater separation distance may be required.

In some production facilities, detection of estrus is practiced by parading the boar past gilt or sow pens. This practice provides very little stimulation to initiate cycling in prepubertal gilts. In any case, a boar used in this manner should be restrained to one group pen or be placed close to no more than 5 individual sow stalls simultaneously for 5 to 10 minutes. This allows the females to realize that the boar is present and for the technician to observe all females exposed to the olfactory cues of the boar. Travel route of the boar should consider air flow patterns in the barn. If observation of all females simultaneously is not possible, a minimum exposure time of 1 hour should be allowed before rechecking with boar exposure to avoid missed detection due to refractoriness.

Type of Mating

All mating systems should be designed around knowledge of physiologic factors previously discussed. Detection of estrus and appropriate timing of insemination are critical. Moving or mixing sows seems to be associated with elevated embryo mortality and should be avoided. Research data on embryo survival per se are lacking, so recommendations are based on the effects of fighting on various physiologic factors believed to affect embryo survival. Available data suggest that if mixing is necessary, the lowest impact on embryo survival will occur if mixing is within 2 days of mating or after 21 days. For practical purposes, delaying moves until after the 42-day heat check enhances efficiency on most farms.

Pen Mating

The use of **pen mating** has declined dramatically in the United States in recent years. The greatest benefit of pen mating is the reduced labor requirements. To maximize efficiency of pen mating requires more management than has been historically applied, with a consequent reduction in this labor advantage relative to that for other types of mating.

Pen mating systems rely on boars to accurately identify and breed sows in estrus. Movement of animals and boar-to-sow ratios can be adjusted to increase the likelihood that this goal will be achieved efficiently. A characteristic of successful pen mating systems is ample boar power, requiring an adequate number of boars, timeliness of use, and sexual rest. A boar-to-sow ratio of 1:8 is common.

Boars should not be expected to breed more than one sow daily. This is achieved by rotating sows into pens with the boar. For example, one or two sows can be added to a pen from each weaning group. Because of courtship behaviors of boars, pen mating can result in some sows not being mated, even though expressing estrus. Using groups of boars seems to reduce this. If new groups are formed after maturity, significant fighting will occur. Rotating boars also is useful. In addition, rotating boars can be coordinated with periods of sexual rest.

Because a majority of weaned sows return to estrus during a relatively short time after weaning, pen mating systems often involve adding sows to groups at weaning to avoid overusing the boar. The risk for reduced embryo survival expected with mixing pregnant sows may not be as dramatic in pen mating systems. Although scientific data are lacking, it appears that the presence of the boar provides an obvious dominant animal, and fighting among sows is dramatically reduced.

Hand Mating

Hand mating describes the practice of taking the female to a breeding pen and housing her with a boar for a

prescribed period of time. During this time the interaction of the animals is observed to allow removal of the female if needed to prevent injury, and to allow assistance with intromission if needed. In practice, very few services should require assistance.

Hand mating has several advantages over pen mating. Boar needs drop to approximately one fourth to one half of those needed for pen mating. Reducing the number of boars brought into the sow unit decreases overall boar cost and the risk of disease introduction. Additionally, greater genetic merit and uniformity of progeny can be expected. Breeding dates are accurately known. Boars that are infertile or subfertile can be detected and treated or eliminated. The disadvantage is that more daily labor is required.

Timing of mating is critical to the success of this program. Detection of estrus is critical to properly timing inseminations. Once- or twice-daily detection of estrus beginning 3 days after weaning is necessary for maximum productivity. Females should remain with the boar for at least 5 minutes. Some gilts and sows require more stimulation before they will exhibit lordosis. After successful mating, the boar should be removed. Ideally the female is allowed to remain in the breeding pen for at least 15 minutes. This is to minimize stress from heat or social interactions. If this is not possible, she should slowly be walked back to her housing area.

With hand mating systems, sows typically are mated once daily at 24-hour intervals for as long as they will stand. This protocol can be modified with *a priori* knowledge of timing of ovulation, as previously discussed. The duration of oviductal sperm viability after natural mating is believed to be longer than after AI, and some farms achieve very high farrowing rates with one natural mating.

Artificial Insemination

Chapter 97 is devoted to AI. Here it is discussed primarily to contrast it to other mating systems. Use of AI skyrocketed in the United States in the late 1990s, and today this method is used to mate a majority of U.S. sows. In comparison with pen mating, the same advantages that apply to hand mating apply to AI, but to a greater degree. The reduction in boar needs is more dramatic, with a 1:150 boar-to-sow ratio routinely achieved. AI also has specific advantages over hand mating. Semen quality can be assessed before use, and infertile or subfertile boars often can be identified before use. The same disadvantages exist. The only additional disadvantage is that a higher level of technical competence is needed to ensure success.

Typical AI programs will use timing and number of matings similar to those with hand mating. Exciting data are being reported using very-low-dose inseminations. These procedures utilize dramatically fewer sperm cells and place the semen into either the uterine body or deep into the uterine horn.[20] Preliminary results suggest 2- to 60-fold more efficient sperm use than with conventional AI, but a higher degree of hygiene and technical competence probably will be required as well.

Boars

The anatomy and physiology of the boar are covered in detail in another chapter of this text. Because the epididymis stores up to 5 days' sperm production, sperm numbers per ejaculate are maximal with 5-day intervals between ejaculation. For AI boars a 5-day interval is recommended for maximum efficiency of sperm collection. Total sperm output can be slightly increased with shorter intervals. For hand mating systems, boars can be used much more frequently. Although some boars will breed two to four times daily, fertility is expected to be lower when mature boars are used to breed more than once or twice daily. Younger boars (<12 months of age) should be used no more than three to five times weekly. After five matings, boars should be allowed a minimum of 24 hours' rest.

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CHAPTER 99

Clinical Examination of Female Reproductive Organs

GLEN W. ALMOND

Detailed examination of records, with or without the assistance of computer programs, often identifies deficiencies in breeding herd performance. The computerized record systems and implementation of statistical process control (SPC) methodology are excellent tools to characterize, at least numerically, specific areas that require long-term or immediate attention. Despite the use of new technology and analytic procedures, swine practitioners often are required to provide accurate diagnoses of noninfectious reproductive failure or urogenital infections in female swine and to identify shortcomings in reproductive performance. Because sow and gilt reproductive organs are easily evaluated at slaughter, this diagnostic technique represents minimal cost to the producer yet provides accurate assessments of the precise causes of reproductive failure.

Complete histories, including age, parity, farrowing dates, weaning dates, and other pertinent information, should be obtained before examination of the reproductive organs. It is necessary to select animals that represent the condition or problem affecting reproductive performance and not animals culled for other reasons. Typically, it is easier to select a greater sample size of animals from sow farms with 1000 or more sows; however, with smaller farms, the entire procedure usually is repeated with two or more groups of animals that typify the farm problem. Reproductive slaughter checks are intended to verify an abnormality that is common to all of the affected animals.

POTENTIAL USES

Clinical examination of reproductive organs is useful to confirm infectious and noninfectious causes of reproductive failure (Box 99-1). Presence of physiologic or anatomic abnormalities associated with reproductive failure can be verified or refuted by examination of the female genital organs. Alternately, findings on these examinations may clearly illustrate shortcomings in breeding management.

Reproductive slaughter checks also can be used for collection of specimens for diagnostic procedures. Fetuses, uteri, oviducts, urinary bladders, and kidneys are readily obtained for histopathologic examination or the procurement of culture swabs. To adequately assess the significance of lesions or other abnormalities, it is imperative that veterinarians have sufficient understanding of normal ovarian and uterine structures.

SPECIMEN COLLECTION TECHNIQUES

Procedures at the Slaughterhouse

Reproductive and urinary tracts are examined at the slaughterhouse or removed to a clinic or laboratory with appropriate approval from regulatory officials. Individual identification of animals at slaughter is used to correlate gross and histologic findings with the reproductive history of the sow. Ear tags are convenient, but ear,

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Box 99-1

Examination of the Reproductive Tract of Female Pigs: Clinical Applications

Noninfectious Causes of Reproductive Failure
Delayed puberty
Failure to return to estrus after weaning (anestrous sows)
"Silent" heats
Regular returns to estrus after mating
Irregular returns to estrus after mating
Pseudopregnancies and not-in-pig sows
Suspected cases of zearalenone toxicity

Infectious Causes of Reproductive Failure
Collection of fetuses and uteri from affected sows before parturition, e.g., PRRSV
Collection of fresh tissue, fetal blood, or thoracic fluid for submission to diagnostic laboratories
Evaluation of reproductive and urinary tracts for evaluation of urogenital infections

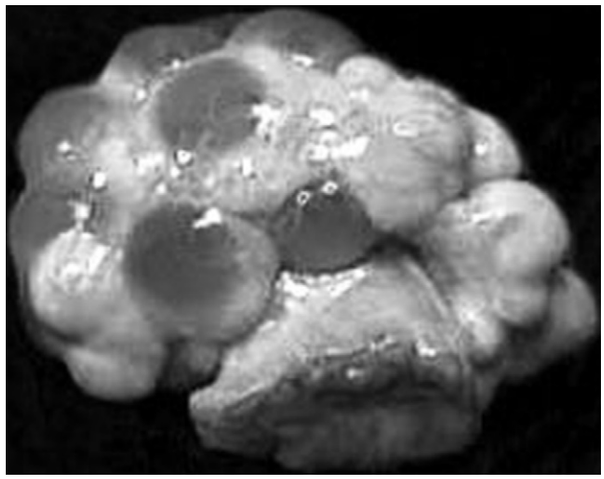


Fig. 99-1 Large, fluid-filled follicles are evident on the ovaries of a sow during proestrus. The corpora lutea are well regressed and white-yellow in color.

shoulder, or flank tattoos constitute a more reliable means of identification.

It is beneficial to examine other body systems while animals are being processed in the packing plant. Body condition and backfat thickness of animals are assessed after evisceration. Hence, additional information is gathered to assist in the interpretation of the cause(s) of reproductive failure. Abnormalities, such as acyclic ovaries, actually reflect suboptimal management of the breeding herd. For example, the absence of backfat on sows during the summer and early autumn months may reflect inadequate feed consumption or facility cooling systems. These shortcomings in management probably contribute to the pathologic conditions noted in the reproductive organs.

If specimens are to be transported to a laboratory, individual sow identification should be included with each specimen. Specimens are placed in sealed containers and packed in ice for transport. It is imperative to tie the cervix and the neck of the bladder closed with heavy string to prevent contamination or leakage.

Initial Procedures and Gross Examination

Gross lesions are noted on examination of the ovaries, oviduct, uterus, cervix, vagina, urinary bladder, and kidneys. If an infectious etiology is suspected, culture swabs are procured using aseptic technique from both horns of the uterus, urinary bladder, and kidneys. Failure to use aseptic technique usually results in gross contamination of the swabs and erroneous culture results. Urine is obtained by centesis with 20-gauge needles and stored on ice (5°C) for transport to the laboratory. Storage of urine for more than 12 hours limits the diagnostic value of the specimen.[1] Owing to the broad spectrum of bacterial agents involved in urogenital infections,[2] swabs are submitted for culture under aerobic and anaerobic conditions. Determination of bacterial sensitivities to antibiotics also should be requested for future reference.

After collection of swabs for bacterial cultures, the vagina, cervix, and each horn of the uterus are incised to allow adequate assessment of the mucosa and luminal contents. The urinary bladder and kidneys also are dissected to examine the bladder lumen and renal pelvis, respectively. To complete the evaluation, it is imperative to collect tissue samples from representative organs for subsequent histologic examination. These samples are fixed in either 10% buffered formalin or Bouin's solution and submitted for histologic preparation and evaluation. If the diagnostic tests may require special handling, storage, or shipping of tissue, it is beneficial to contact the diagnostic laboratory before collection of samples.

NORMAL MORPHOLOLGY

Normal Ovarian Structures

The possibility of errors in the recording of a sow's reproductive history make it necessary to determine the stage of the estrous cycle during gross examination of the ovaries. In addition, interpretation of uterine morphologic features is improved when the stage of the estrous cycle is known. The estrous cycle of the pig typically ranges from 17 to 25 days. Owing to this variation in the length of the estrous cycle, it is more convenient to classify the cycle into three distinct phases; proestrus (follicular phase), estrus (sexual receptivity), and diestrus (luteal phase).

Follicle Growth and Ovulation

Ovarian follicles destined for ovulation grow from approximately 4 to 5 mm in diameter on day 15 (day 0 = the first day of standing estrus) of the estrous cycle to an ovulatory diameter of 8 to 12 mm (Fig. 99-1). Preovula-

tory follicles have taut, almost transparent walls and contain straw-colored fluid. Estrogen (17β-estradiol), the predominant ovarian hormone produced at this time, contributes to estrous behavior and to morphologic changes in the reproductive tract and triggers the surge of luteinizing hormone (LH) from the anterior pituitary gland.

The LH surge is necessary to induce ovulation or rupture of the follicles with the subsequent release of ova. Onset of estrus usually coincides with the preovulatory LH surge; depending on age, females may be sexually receptive for 1 to 3 days. Ovulation occurs in most gilts during the latter part of the second day after the onset of estrus; however, the time between the onset of estrus and ovulation varies considerably among sows.

Luteal Structures

After ovulation, collapsed follicles are 4 to 5 mm in diameter. Blood rapidly fills the central cavity of the follicles. At this stage, these blood-filled structures are considered corpora hemorrhagica. Luteinization of the follicular remnants results in the formation of multiple corpora lutea. By day 5 to 6, each corpus luteum (CL) has reached its mature diameter of 9 to 11 mm, and the central cavities are completely replaced by luteal tissue (Fig. 99-2). The corpora lutea produce the steroid hormone progesterone. Serum progesterone concentrations, detectable within 1 to 3 days after estrus, increase until a maximum is achieved during mid- to late diestrus.

Degeneration of the corpora lutea commences at approximately day 13 to 15, coinciding with increased concentrations of the luteolysin prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)[3] and decreasing concentrations of progesterone. It is assumed that $PGF_{2\alpha}$ contributes to regression of the corpora lutea; however, other factors evidently influence the structural regression of the corpora lutea. Obvious indications of CL regression are the change in color from reddish-pink to yellow and a gradual decrease in diameter. A new wave of follicular recruitment commences on days 13 to 15, with follicle enlargement and hyperemia obvious by day 17 (Fig. 99-3). Follicles continue to enlarge concomitant with regression of the corpora lutea.

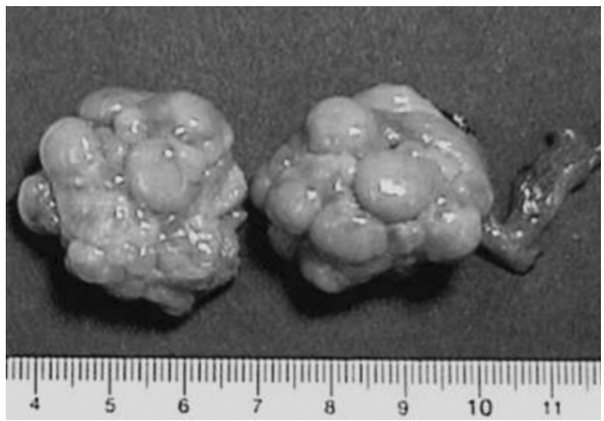
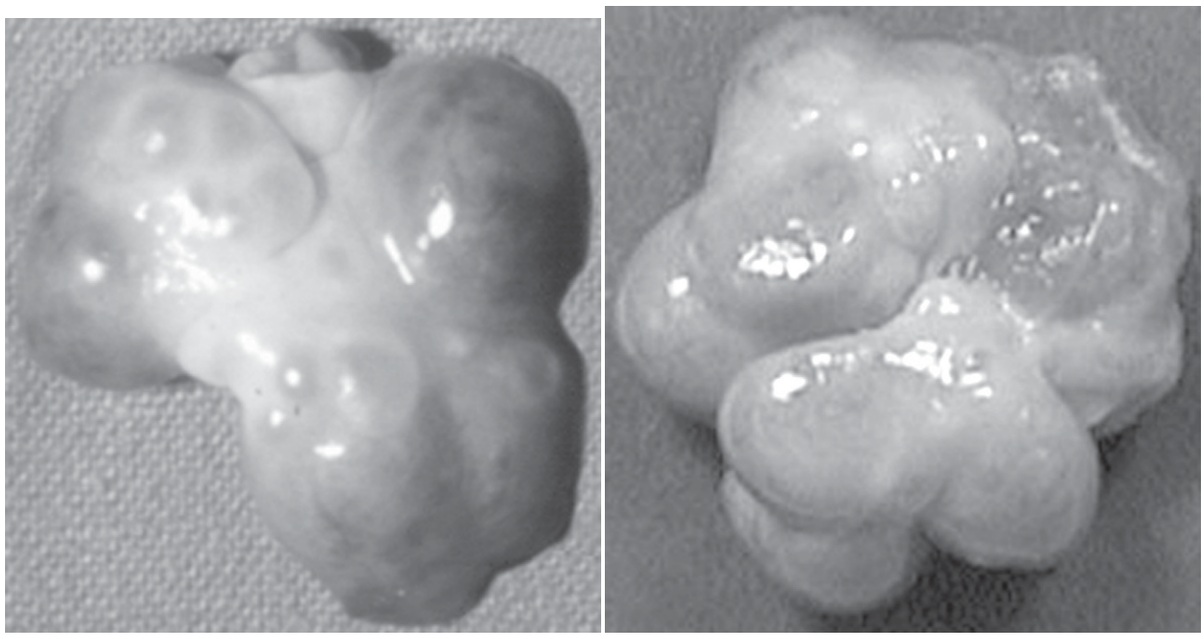


Fig. 99-3 Ovaries collected from a sow that was in late diestrus to early proestrus. The corpora lutea are beginning to regress. By contrast, growth of follicles has commenced, and follicles are approximately 5 mm in diameter.



A **B**

Fig. 99-2 **A,** Ovaries collected from a sow at 1 to 2 days after ovulation. The blood-filled structures are corpora hemorrhagica. **B,** Mature corpora lutea are evident on the ovaries of a sow during diestrus.

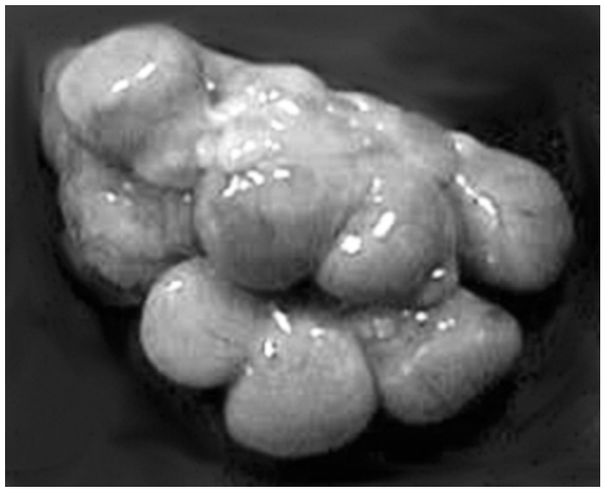


Fig. 99-4 During pregnancy, the corpora lutea are similar to those in sows during diestrus. The corpora lutea of pregnancy are pale compared with those of diestrus.

With maternal recognition of pregnancy (occurring on days 10 to 14 after ovulation and successful mating),[4] CL lifespan is extended throughout pregnancy. The gross appearance of the corpora lutea of pregnancy (Fig. 99-4) is remarkably similar to that of the corpora lutea during diestrus. Dissection of the uterus will reveal fetuses if the animal's pregnancy had progressed to 20 days of gestation or later. Before 20 days of gestation, it is useful to flush both horns of the uterus to detect the presence of early embryos. Confirmation of pregnancy status is particularly applicable when the quality of matings or estrus detection requires scrutiny or when records of these events are not maintained in an orderly and detailed fashion. It is not uncommon to observe pregnancies in animals that were erroneously recorded as anestrous. These observations illustrate weaknesses either in the record system or in management practices.

After parturition, the corpora lutea regress to form corpora albicantia. These "white bodies" gradually decrease in size during lactation and are less than 2 mm in diameter at the time of weaning. Progesterone production is minimal by the corpora albicantia during lactation. Follicular development is inhibited during lactation, and few follicles are greater than 4 mm in diameter. Limited follicle growth occurs in late lactation (beyond 2 weeks after farrowing)[5]; however, the number of follicles greater than 5 mm in diameter increases within 2 to 3 days after weaning and with removal of the inhibitory effects of lactation. Follicles continue to increase in size as the animal approaches estrus after weaning.

Normal Morphology of the Tubular Organs

The tubular components of the genital organs include the vestibule and vagina, cervix, uterine horns, and oviducts. The gross appearance and histologic morphology of these organs change in a dynamic fashion during the estrous cycle. The primary determinant of these cyclic changes is ovarian production of estrogen and progesterone. Therefore, changes in ovarian structures and function exert corresponding changes in the tubular components of the tract.

Vagina and Vestibule

The vagina of the female pig is approximately 7 to 12 cm (3 to 5 inches) in length and is the part of the reproductive tract extending from the cervix to the urethral orifice. The vestibule is located caudal to the urethral orifice and occasionally is termed the caudal vagina. The vestibule is 7 to 9 cm (3 to 4 inches) in length and has numerous longitudinal folds, two rows of small round openings (minor vestibular glands), and solitary lymph nodules.

The predominant cell types in the epithelium of the vagina are either low cuboidal or stratified squamous cells. Changes in the vaginal epithelium tend to lag from 1 to several days behind ovarian changes, with considerable individual variation. Rapid growth of the vaginal epithelium occurs under the influence of estrogen. In addition, the vulva of the pig swells and has increased turgidity during periods of high serum estrogen concentration. The swelling subsides as estrogen concentrations decrease and the animal enters so-called standing estrus. Decreasing estrogen concentrations result in the degeneration and sloughing of the numerous epithelial cell layers from the vagina and the formation of vacuoles that contain dead cells and a few leukocytes.[6] By contrast, low cuboidal epithelium (4 to 6 layers) is present when progesterone concentrations are elevated—namely, during pregnancy, pseudopregnancy, and the luteal phase of the estrous cycle.

Cervix

The porcine cervix may be up to 20 cm (8 inches) in length and contains obvious transverse ridges. The lumen is lined with tall columnar epithelial cells and numerous goblet cells. The goblet cells have a large secretory surface and secrete mucus during the estrogenic phase of the cycle. Estrogen also stimulates contraction of the sphincter muscle and tightening of the lumen. During the luteal phase of the cycle, progesterone causes relaxation and closure of the cervix.

Uterus

The uterine wall consists of three layers: (1) the endometrium, consisting of the epithelial lining of the lumen, the glandular layer, and the connective tissue layer; (2) the myometrium or muscle layer, which is highly vascular; and (3) the serous membrane. The uterine glands are tubular invaginations of the epithelium and are either straight or convoluted. As a result of estrogen stimulation in the follicular phase, the glands are simple and straight, whereas the epithelium is lower and the endometrium thinner. By contrast, increased vascularization is present in the muscle layer, resulting in greater weight and tone of the uterine horns.

The endometrium increases in thickness under the influence of progesterone during the luteal phase. This increase reflects the branching and convolution of the

glands. Vascularity and uterine weight are reduced considerably during the luteal phase.

Oviducts

The basic functions of the oviducts are to "trap" the ova at ovulation, to transport the ova to the ampulla-isthmus junction (site of fertilization), and to provide a suitable environment for the ova, sperm, and early embryos. The lumen of the oviduct is lined with a folded mucous membrane with a ciliated, simple columnar epithelium. The isthmus has longitudinal folds that extend to the tip of the uterine horn. The folds are arranged as polyploid processes that are sensitive to ovarian steroids, particularly estrogen, which causes swelling of the processes during the preovulatory phase of estrus.[7] The uterotubal junction of estrous pigs is not patent to the passage of fluids from the uterus to the oviduct.

Because of the restricted luminal size of the oviduct, it is difficult and time-consuming to assess patency with routine dissection. To determine oviduct patency, a 20-gauge needle is inserted through the uterotubal junction, and 5 to 10 ml of saline is infused through the needle into the oviduct. The saline should pass through the oviduct and fill the infundibulum around the ovary. If subsequent histologic assessment of the oviducts is required, formalin is used for flushing. Use of this agent assists fixation and prevents sloughing of epithelial cells in the oviduct.

ABNORMALITIES OF THE REPRODUCTIVE TRACT

Ovarian Abnormalities

Cystic Ovaries

Multiple large, multiple small, and single cysts occur in the ovaries of sows[8]; however, the behavior and physiologic events differ between animals affected with each type of cyst. Most of the multiple large cysts (Fig. 99-5) have some luteinized tissue and produce sufficient progesterone to inhibit estrous cyclicity. Sows with such cysts usually fail to exhibit estrus and are culled from the herd. By contrast, multiple small cysts often are follicular cysts. Estrogen is the predominant steroid produced by follicular cysts, and affected animals may have irregular estrous cycles or exhibit "nymphomania." Because these animals fail to ovulate, it is unlikely that they will conceive. Single ovarian cysts rarely affect fertility or the estrous cycle of sows; their presence is noted in occasional sows at slaughter. Cystic corpora lutea evidently regress with the normal corpora lutea and supposedly do not interfere with fertility.

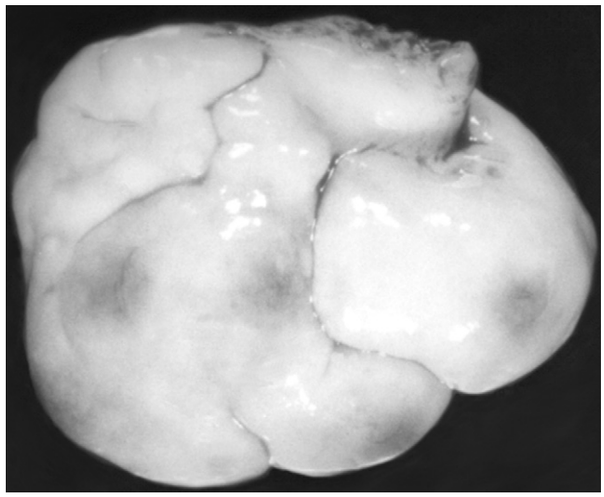


Fig. 99-5 This photograph illustrates multiple large cysts on the ovary of a sow. Note the thickened wall of the cyst. Typically, these cysts have luteinized tissue, which produces progesterone.

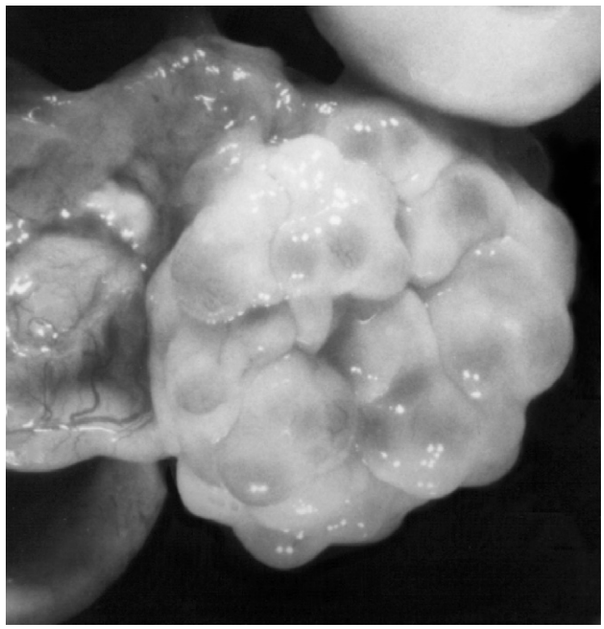


Fig. 99-6 Ovaries collected from an anestrous sow at 20 days after weaning. Note the absence of corpora lutea and presence of well-regressed corpora albicantia. Follicles are less than 5 mm in diameter.

Acyclic Ovaries

Anestrous sows are animals that fail to return to estrus following weaning. Ovaries from these sows have small follicles (less than 5 mm in diameter), well-regressed corpora albicantia, and no corpora lutea (Fig. 99-6). As previously mentioned, ovarian changes are minimal during lactation. Therefore, the gross appearance of ovaries from anestrous sows and that of ovaries from lactating sows essentially are identical despite differences in the endocrine mechanisms that inhibit follicle growth and maturation.

Ovaries from prepubertal gilts weigh approximately 0.3 g at 70 days of age. The weight of both ovaries will increase to 5.5 g at 112 days and stay at this weight until puberty.[9] Few follicles are greater than 3 mm in diameter at 140 days of age. In gilts with delayed puberty, findings typically include numerous small follicles (less than 4

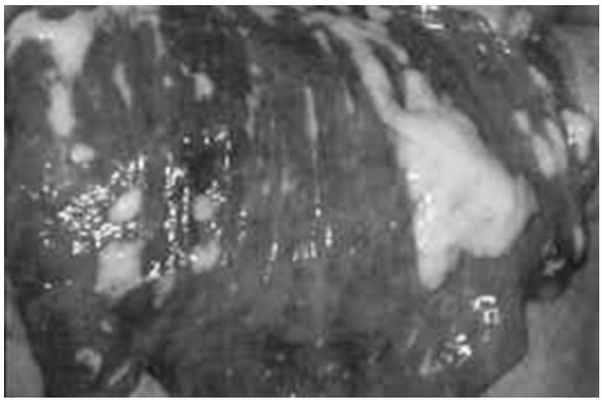


Fig. 99-7 The reproductive tract collected from a "discharging" sow. Note the large accumulations of purulent debris on the endometrium. Typically, purulent material is difficult to observe in sows with endometritis or metritis.

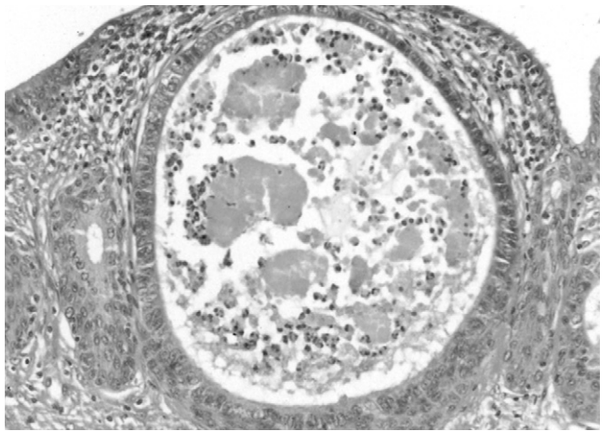


Fig. 99-8 This photomicrograph illustrates a microabscess in the vagina of a sow during estrus.

to 5 mm in diameter) without corpora lutea or corpora albicantia (see Fig. 99-6). Along with the failure of ovarian development, growth of the uterus and oviducts is retarded. The uterus typically weighs approximately 180 g and the oviducts are about 22 cm in length at puberty.[9] Obviously, a gilt with delayed puberty has acyclic ovaries and poorly developed uterus and oviducts.

Abnormalities of the Uterus, Oviducts, and Cervix

Metritis and Endometritis

Vulvar or vaginal purulent discharges commonly are observed in gilts and sows at 10 to 18 days after mating. It is assumed that these discharges originate from the uterus and are indicative of uterine infections.[10] **Metritis** is inflammation of multiple layers of the uterine wall, whereas **endometritis** refers to inflammation of the uterine lining or endometrium. It is unusual to observe metritis on histologic examination of the uterus from affected sows; a majority of animals have endometritis.

The contents of affected uteri vary widely, reflecting the pathogenic organism, chronicity of infection, and the stage of the estrous cycle.[10] Uteri may be distended with debris and exhibit thickening of the wall (Fig. 99-7), and uterine contents vary, ranging from vast quantities to focal accumulations of purulent exudate. By contrast, the uteri of many affected sows are devoid of debris after a standing estrus. Finally, in many instances, normal vulvar discharge associated with estrus is erroneously classified as indicative of metritis or endometritis. Gross and histologic examination of the reproductive organs will clearly illustrate the source of the discharge.

Salpingitis

The incidence of salpingitis, with or without endometritis, is essentially unknown in female pigs. Early reports indicated that salpingitis was associated with porcine reproductive inefficiency[11]; however, recent investigations did not examine the oviducts of sows affected with endometritis.[12] Histologic examination of serial sections of the oviduct would be useful to evaluate salpingitis. Oviduct patency is easily determined, as described previously. Adhesions of the infundibulum to the ovary occasionally are noted in sows without endometritis. Possibly, hemorrhage associated with ovulation or the rupture of ovarian cysts contributes to the infundibular adhesions.

Cervicitis

The significance and pathogenesis of cervicitis in female pigs have received little attention. One report indicated that a modest vulvar discharge (less than 20 ml in amount) occurs in animals with cervicitis and typically is concomitant with endometritis or vaginitis.[2] Gross examination of the cervix, with subsequent histologic assessment, should confirm the purulent nature of the exudate and presence of cervicitis.

Trauma to the cervix may result in the formation of fibrotic adhesions, which may occlude the lumen. Historically, sexually aggressive boars were considered the most likely cause of trauma to the cervix. The use of artificial insemination has gained popularity in the United States, however, and it is not uncommon to note hemorrhage and subsequent formation of cervical lesions after insemination by an inexperienced or impatient technician.

Vaginitis and Vestibulitis

Vaginal infections previously were reported to occur with or without chronic infections of the uterus or urinary tract.[2] Regardless of whether such infection is present, hyperemia and congestion, hemorrhage, and purulent exudate are typical of vaginitis. Microabscessation (Fig. 99-8) and erosions or ulcerations of the mucosa may be noted on histologic examination.[2] Similar lesions are evident in the vestibule.

Zearalenone Toxicity

Zearalenone, a toxin produced by *Fusarium roseum,* acts like estrogen in the sow. Consequently, affected sows

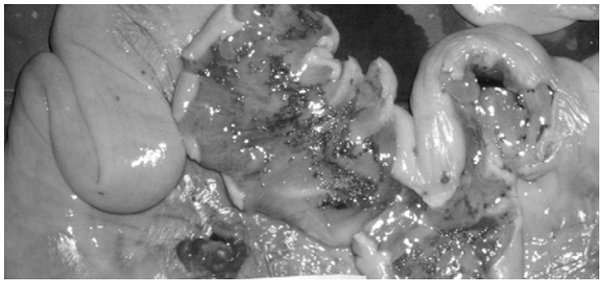


Fig. 99-9 With chronic exposure to estrogen or zearalenone, the endometrium becomes edematous and the uterine glands secrete uteroferrin. In this figure, the uteroferrin is evident as brown flecks and should not be mistaken for purulent debris.

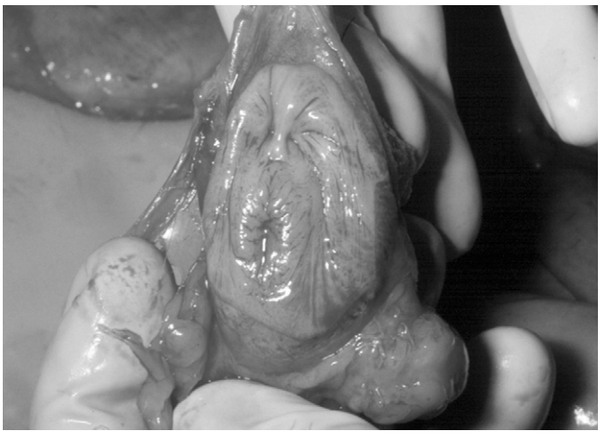


Fig. 99-10 Hymenal bands (dorsoventral septa) in the vagina are common in virgin gilts. In this example, the band is cranial to the urethral orifice and obstructs the cervical os. The width of the bands is quite variable.

have excessive uterine edema and numerous brown flecks (1 to 2 mm) of glandular secretions (uteroferrin) occasionally are noted on the endometrium (Fig. 99-9). These animals often exhibit estruslike signs, such as a red, swollen vulva; however, the ovaries do not contain mature, preovulatory follicles. Paradoxically, the ovaries often have corpora lutea because estrogen is luteotrophic in female pigs, and it is impossible for the animal to conceive.

Miscellaneous Abnormalities

Various other developmental abnormalities may be noted in the genital tract of gilts. Blind uterine horns, unilateral missing uterine horns, and blind, double, or missing cervix are relatively rare abnormalities (seen in less than 1% of gilts at slaughter).[13] Persistent vaginal hymen or presence of hymenal bands (dorsoventral septa) (Fig. 99-10) previously was observed in 4% of gilts; however, the clinical significance of these bands for gilt fertility remains unknown.

EMBRYONIC AND FETAL AGE

Recording of an erroneous breeding date and failure to record the breeding date are common on sow farms. Consequently, veterinarians must rely on other techniques to estimate the age of embryos or fetuses during examinations of the female reproductive organs.

The uterus is flushed with 100 to 200 ml of saline to collect blastocyst or morula stage embryos. Examination of flushings with a dissecting microscope facilitates detection of embryos less than 8 to 9 days of age. Older embryos typically are visible without the assistance of a microscope. Elongation of the threadlike blastocysts is evident by day 13 of pregnancy, and attachment of the trophoblast to the endometrium commences by day 13 and is well advanced by day 18.[14] Although embryo attachment is initiated at this early stage of pregnancy, routine flushing of the uterus should dislodge the embryos without excessive difficulty. The embryonic sacs range from 18 to 35 cm in length (in situ) during the period from days 13 to 18; however, it often is difficult to observe the embryos in situ.

Crown-rump length of fetuses provides a reasonable estimate of fetal age after day 20 of gestation. At days 25, 35, 45, 55, 65, 75, 85, and 95 of gestation, values for fetal crown-rump length are approximately 20, 35, 65, 110, 152, 166, 206, and 240 mm, respectively.[15] Number of fetuses, infectious microbes, parity, genetics, and other factors may influence relative size of some or all of the fetuses. This approximate determination of fetal age is useful to assess accuracy of breeding records, to estimate age of mummified or autolyzed fetuses, and to determine the time of fetal death in relation to stage of gestation.

The number of corpora lutea reflects the number of ovulated follicles, thereby providing an indication of the number of ova released at ovulation (i.e., ovulation rate). Quantitation of embryos at days 14 to 18 of pregnancy is useful to estimate early embryonic loss, whereas the number of fetuses in late gestation reveals both embryonic loss and early fetal loss before fetal calcification. It is not uncommon to note a 30% to 40% rate of early embryonic loss in most domestic sows.

CYSTITIS

Cystitis often is associated with reproductive tract infections, particularly in sows affected with purulent vulvar discharges. Collection and examination of urinary bladders are easily performed during evaluation of the genital organs. As previously mentioned, urine samples and mucosal swabs should be collected with aseptic techniques before dissection and examination of the mucosal surface of the bladder.

Mucosal hyperemia and congestion, mucosal ulceration, and accumulation of fibrinopurulent exudate over affected areas are indicative of cystitis.[2,16] The method of stunning in the slaughterhouse must be considered in interpreting the hyperemia and congestion in the

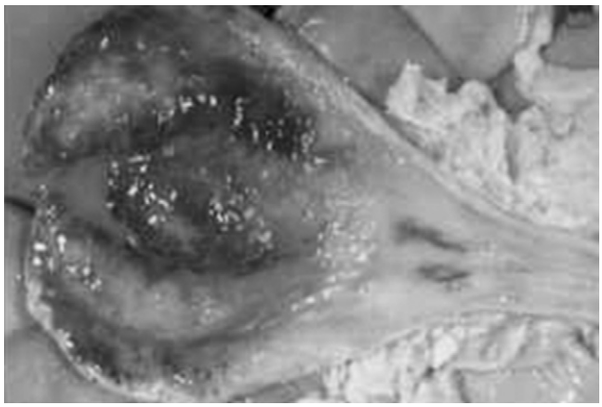


Fig. 99-11 This figure illustrates a urinary bladder with severe cystitis. The hemorrhagic areas and purulent urine are clearly evident. Hemorrhage and necrosis of the ureteric valves often are noted in severe cases of cystitis.

bladder. Prolonged electrocution may contribute to vascular changes in the bladder at the time of death. Thickening of the bladder wall and polyploid projections of the mucosa are apparent in chronic cystitis. The relationship between cystitis and pyelonephritis and respective lesions were reviewed elsewhere.[2]

Ureteric valves and intravesicular ureters deserve special attention because abnormalities of these structures are common in animals affected with pyelonephritis[17] (Fig. 99-11). Enlargement of the ureter and necrosis of the ureteric orifice were observed in animals with acute pyelonephritis, whereas the orifice was shrunken and swollen in animals affected with chronic pyelonephritis. Bacteria-produced factors evidently contribute to shortening of the intravesicular portion of the ureter in cases of acute and chronic pyelonephritis.[17]

SUMMARY

In contrast with the cow or mare, it is difficult or impossible for the veterinarian to examine the genital organs of a live sow or gilt. Therefore, detailed examination of the genital organs of female pigs at slaughter or necropsy is required to provide sufficient information to confirm or refute causes of reproductive failure. Consideration must be given to individual sow characteristics and herd history to improve the practitioner's interpretation of the lesions or abnormalities. The appropriate therapy or management changes can be suggested with a greater degree of confidence after evaluation of the reproductive organs of affected animals.

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CHAPTER 100

Induction of Estrus and Control of the Estrous Cycle in Swine

ROBERT V. KNOX and WARREN D. WILSON

Despite many years of research investigating pharmacologic methods to control reproduction, swine producers and veterinarians are left with few options compared with those available for use in cattle. Nevertheless, the pharmacologic and managerial methods that have been developed have allowed for improved reproductive performance. Even so, estrous cycle control may not be practical or desired in all groups of swine, and pharmacologic methods must not be used to replace basic swine husbandry practices. Yet clear advantages are realized for swine producers who are able to control the timing of estrus and ovulation in large numbers of sows and gilts. Reproductive efficiency and improved economic return can be attained through maximizing the number of piglets weaned per sow per lifetime, reducing the number of nonproductive sow days, optimizing facility utilization for gilts, and reducing labor inputs. Although hormonal availability for control of pig reproduction varies throughout the world, most pharmacologic methods are similar and involve initiation of final follicular maturation, alteration of luteal lifespan, or synchronization of ovulation time. This chapter reviews the current knowledge of pharmacologic and management methodology for optimizing control of reproduction in the pig.

THE PREPUBERTAL GILT

Understanding the endocrine profile of the prepubertal gilt holds significance for influencing the timing of puberty and for developing technologies for inducing early reproductive function. Reproductive hormones originating from the hypothalamus, pituitary, and ovary regulate reproduction. Although hormones are synthesized and released by positive and negative feedback relationships in the mature female, in the prepubertal gilt the endocrine organs and their responsiveness to feedback are limited. Maturation is gradual and dependent on internal and external influences, some of which have yet to be identified. Endocrine control of ovarian activity occurs primarily through the pituitary hormones follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones control the processes of folliculogenesis and ovulation through their binding to receptors on the granulosa and theca cells of the ovarian follicle. Hormone binding stimulates a variety of activities including cell division, steroid synthesis, and follicle growth. Both hormones are controlled by hypothalamic gonadotropin-releasing hormone (GnRH) and by positive and negative feedback from ovarian steroids and the inhibin family molecules.

Immediately after birth, FSH, LH, and prolactin levels are elevated in circulation, but they gradually decline with age. This elevation is thought to occur because production mechanisms are functional, but feedback regulation has not yet developed. By contrast, circulating estrogen is low at birth but gradually increases with age in association with the increase in size and number of antral ovarian follicles.[1] The hormone profile before pubertal estrus and during the subsequent cycle is not different from that in later cycles; accordingly, endocrine indicators of why gilts become reproductively active have been elusive. Immediately before pubertal estrus, however, plasma cortisol and prolactin are elevated, but these increases do not appear related to age at puberty. In gilts expressing first estrus between 183 and 225 days, progesterone has not been observed to be consistently elevated within the 6 days before pubertal estrus. No increase in estradiol occurs in the weeks preceding pubertal estrus except for the expected increase in estrogen that occurs before estrus. Of interest, some gilts have significant estrogen increases that last for days after exposure to a boar, but most fail to show estrus and have no detectable LH surge. The external symptoms of estrus such as vulvar swelling and mucous secretion are inducible at 150 days of age with exogenous estradiol. Sensitivity to estrogen may be underdeveloped in immature gilts, however, because low doses that produce physiologic symptoms of estrus in adult females are only partially effective in prepubertal gilts, despite the fact that all gilts exhibit an LH surge. LH level remains basal until the ovulatory LH surge at estrus. Therefore, attempts to advance age at puberty in the gilt by exogenous administration or selection of the aforementioned hormones probably are of little benefit.

Factors Influencing Attainment of Puberty in the Gilt

Gilts that express early puberty and are mated earlier produce more pigs per lifetime than those mated at later ages. Unfortunately, age at puberty can vary by 60 to 80

days for gilts that express estrus before 300 days of age. The factors influencing age at attainment include genetic composition.[2] Differences within purebreds for age at puberty, however, can be as great as differences between breeds, owing to the different selection pressures from different genetic suppliers over time. It therefore becomes difficult to select purebred lines that have uniformly reduced age at puberty. Yet one of the most striking effects of breed on age at puberty is observed for the Meishan gilt, which reaches puberty at approximately 100 days of age, compared with 210 days for occidental breeds. Furthermore, offspring from matings of Meishan sires with crossbred occidental maternal line gilts show reduction in age at puberty and improved percentage of gilts expressing estrus, compared with females sired by occidental-breed boars.[3] Despite these important observations, a majority of commercial maternal line genetics used today are the result of occidental multiple-breed crosses, and age at puberty in these lines indicates no advantage for reduced age at puberty.[4] This finding is perhaps not surprising because most selection schemes for maternal lines do not include age at puberty.

Direct selection for reduced age at puberty has produced an estimated heritability between 0.11 for a single generation and 0.25 over multiple generations. Age at pubertal estrus can be reduced by –1.5 to –3 days in each generation with selection.[5] Selection for age at puberty is desirable and is not associated with any reduction in litter traits. Research also has shown expression of early puberty is not dependent on boar exposure because early puberty lines lacking boar exposure still express earlier puberty compared with nonselected lines.[6]

The available information is conflicting regarding the importance of gilt body composition and its relationship to puberty. Liveweight explains 77%, tenth-rib fat 40%, and average daily gain less than 10% of the variation in age at puberty.[7] It has been reported that gilts with more backfat thickness at 90 kg have more intense vulvar reddening and swelling at estrus, and gilts with strong vulvar symptoms are younger and lighter at estrus.[8] Similarly, in selection experiments, gilts selected for early puberty also tend to cycle earlier and have more backfat than those gilts selected for later puberty. Therefore, selection for age at puberty and related traits of economic importance must become a greater priority in reproductive selection processes.

It is difficult to separate out the effects of nutrition from growth rate and body composition. Nutritional influences, however, can greatly influence the genetic potential to express growth and body composition traits. Of the numerous reviews on the effects of diet for the developing gilt, most agree that severe energy or nutrient restriction can delay or even prevent puberty, whereas minor restrictions may have little detrimental effect on measured reproductive traits. Furthermore, excess energy intake also has not improved reproductive performance. Increased protein, and specifically, certain amino acids, has resulted in some cases of improved reproduction, however. This infrequent outcome may result from the complex issues of amino acid ratios, protein-to-energy ratios, and variation in reproductive responses measured. Nevertheless, at present most experts agree that when energy is not limiting and protein is available for maximal lean tissue accretion, diet should not be limiting to reproductive performance.

Physical and physiologic maturity are reported to be more important for attainment of puberty than age, weight, and even boar exposure.[9] Of all identifiable factors, physiologic age appears to hold the greatest relationship to the age at attainment of puberty. Physiologic age, more than chronologic age, is important because even though gilts can ovulate in response to exogenous gonadotropins and respond to exogenous estradiol with an LH surge after 100 days of age, strong evidence indicates that neither of these mechanisms is completely mature until after 160 days. This finding indicates that induction procedures should not be initiated too early.

The effect of season of birth on puberty is difficult to separate from the effects of temperature, light, and changing photoperiod during development. Some researchers, however, have shown that developing gilts during a shortening photoperiod (fall-winter) and maturing them under increasing light (spring-winter) induces a greater proportion of the animals into puberty than is observed with development of gilts during increasing photoperiod (spring-summer) and maturation under shortening photoperiod (fall-winter). Unfortunately, little evidence is available to suggest an overall advantage in earlier puberty by lighting regimen or type of light. Excessive darkness does delay estrus, however, and continuous light does not advance age at estrus when compared with shorter 8- to 16-hour intervals of light each day.

Although the issue is controversial, confinement has been shown in some cases to increase the proportion of anestrous gilts and to delay age at puberty by 3 weeks.[10] The general consensus is that that gilts raised in restricted space may be less likely to express estrus at an earlier age. Mature gilts raised in only 1 square meter of space expressed estrus at a lower rate than that observed for animals allowed 3 square meters of space (79% versus 100%).[11] The importance of small group size is unclear, but housing developing gilts in groups of less than 3 should be avoided, and little evidence is available to suggest an effect of housing 3 to 30 pigs per pen on age at puberty.

Boar exposure is an effective method for reducing age at puberty, inducing estrus, and improving estrus synchrony. The effect of boar exposure is presumed to be physiologic because it has been shown to transiently increase both LH and estradiol concentrations.[12] Exposing gilts to boars between days 150 and 170 appears to be most effective for advancing age at puberty and results in the highest degree of estrus synchrony. Earlier boar exposure beginning at 70 days of age, when compared with no boar contact or boar exposure at 160 days of age, had no effect on advancing age at puberty, however. Exposure duration of 5 to 30 minutes each day appears equally effective for advancing age at puberty.[13] Yet the proportion of females reaching puberty within 60 days from initiation of boar exposure at 160 days of age does improve as frequency increases between one and three times per day.[14] Continuous boar contact appears to have little detrimental effect on age at puberty, except that it can make detection of estrus in gilts more difficult.

Greater response to boar exposure can be obtained when combined with transportation stress at 160 days. This regimen shows major response effects for boar contact alone but not for transport alone. Typically, the boar effect is not observed in the first week but is evident by 30 days. Transportation for 1 km or more in conjunction with boar exposure has the greatest impact on the proportion of females induced into puberty.[15] To obtain optimal responses for estrus induction, physical contact with the boar is now recommended as superior to fenceline contact.[16] Age of the boar also is important, because exposure to boars 11 months of age and older is more effective than exposure to those 6.5 months of age or younger. It also is unclear whether exposure to a second boar or exposure of gilts to estrous sows will be effective for influencing the proportion of gilts expressing estrus, even though age at puberty may be reduced.[17]

It is not known whether ovarian status affects age at puberty. Ovary classification before puberty at 150 days of age and at 5-day intervals thereafter shows ovaries with only small and those containing larger follicles.[18] Of interest, the ovaries switched from one type to another over time, and no changes in circulating hormones were associated with any of these ovarian class changes. This evidence may suggest that follicle growth occurs in waves and that larger follicles in the prepubertal gilt undergo regression and do not undergo ovulation before puberty. The gilts with large follicles greater than 6 mm in diameter had a greater number of ovulations when compared with those with smaller follicles in response to human chorionic gonadotropin (hCG) given at 170 days of age.[19] Ovaries containing larger follicles versus those with only small follicles, however, do not always show an overall advantage in response to gonadotropins on estrus induction response, even though an early puberty line with only large follicles at time of injection did respond at a higher level than the nonselected line containing large follicles.[20] One explanation for this finding may be the observation that at 166 days in Large White gilts, only half of them have follicles (greater than 3.5 mm) that are responsive to hCG.[21] Thus, differences in physiologic maturity appear to be regulated at the origin of the ovary and may explain why only some animals respond to exogenous hormones.

Exogenous Hormone Control

In the pig, equine chorionic gonadotropin (eCG), also called pregnant mare's serum gonadotropin (PMSG), is the most commonly used and most effective product for induction of follicular growth, estrus, and ovulation in swine. In many European countries, eCG is commercially available for inducing estrus in sows and gilts. The hormone is administered in doses ranging from 250 to 2000 IU but often shows mixed results for estrus and ovulation, which may depend on the preparation purity, dosage used, and even physiologic status of the animal. Use of even moderate to high doses of eCG (greater than 750 IU) has not consistently resulted in improvements. Another gonadotropin, hCG, also will induce ovulation, but it is relatively ineffective in initiating estrus. When administered alone (in a dose of 200–750 IU), however, hCG has been reported to induce estrus and ovulation in prepubertal gilts, but as with eCG, this response also appears highly variable.

A combination of eCG and hCG has proved to be even more effective than either hormone given alone. In studies that have compared the use eCG and hCG alone with the administration of the hormones simultaneously or the use of 500 IU of hCG at 48 to 96 hours after an injection of eCG, estrus expression, ovulation, and ovulation rate all are improved. The most efficient and effective dose appears to be 400 IU of eCG and 200 IU of hCG, although variation of the amount of eCG (300–1000 IU) and hCG (200–750 IU) in a single injection or given separately 48 to 72 hours apart has been reported to be effective. Despite this, little or no advantage is gained with use of higher doses of either hormone. In most instances, the low doses of eCG (less than 750 IU) induce low to normal ovulation rates (6 to 16 ova), and superovulation (greater than 20 ova) usually is induced only after a dose of 1000 IU or more. The use of these agents combined in a single injection (400 IU eCG and 200 IU hCG) was made possible by the development of PG600.* This combination hormone is approved worldwide for induction of puberty in gilts that have attained a weight of 185 pounds and an age of $5^1/_2$ months. In most published reports, PG600 has been effective for inducing greater than 55% of gilts into estrus within 7 days of injection. The percentage of gilts ovulating is greater than 90%, and average ovulation rate, although variable, typically falls between 12 and 15 ovulations. When inducing puberty in gilts after 180 days of age and mating at the induced estrus or even at a fixed time after administration of PG600, 65% to 70% farrow, with no changes in litter size observed. When one cycle is skipped before breeding after PG600 estrus induction, however, both litter size and farrowing rate improve.[22]

Hormone products other than eCG (with or without hCG) are not as efficacious in inducing estrus and ovulation. Single injections of 250 μg of GnRH consistently fail to induce ovulation or estrus in gilts because the short release of FSH and LH apparently will not induce estrus or ovulation. Injections of GnRH in addition to eCG have not improved the response. Hourly administration of GnRH for several days is required to induce estrus and ovulation with this hormone alone. Combinations of porcine FSH and LH also have been reported to be effective for inducing estrus and ovulation, but their short half–lives (approximately 0.5 to 1 hour) relative to that of eCG (36 hours) dictates that they be administered in large doses and at multiple intervals. Induction of estrus in prepubertal gilts by estrogen administration (5–200 μg/kg) in single or even multiple injections over 2 to 3 days has resulted in both good and poor induction rates. In some instances, however, estrus without ovulation and low numbers of ovulations are observed. Exogenous estrogens such as estradiol benzoate (17β-estradiol-3-benzoate), and estradiol cypionate (estradiol-17β–cyclopentylpropionate) will consistently induce estrus and a preovulatory-like discharge of LH but will

*Intervet, Millsboro, Del.

induce ovulations in only a few instances. When females are treated with hCG plus estradiol, both estrus and ovulation occur, but litter size is reported to be reduced. As indicated by the relative ineffectiveness of these alternative hormones in comparison with eCG and hCG, and the difficulty in administration, these methods become less than practical in commercial situations.

CYCLIC GILTS AND SOWS

The control of reproduction in the mature cycling gilt and sow is limited to the techniques of superovulation through administration of hormones such as eCG, shortening or lengthening the estrous cycle with prostaglandin, use of supplemental progestogens or estrogens, and controlling the time of ovulation through the use of hCG, LH, or GnRH near the time of estrus. Cyclic gilts administered porcine FSH or PMSG at luteolysis express estrus sooner, but many fail to express estrus at all, owing to asynchronous follicle development and formation of cysts. In those that do express estrus, ovulation rate typically is much greater, however. Breeding after superovulation increases the number of embryos but generally does not improve litter size, owing to limitations in uterine capacity. Controlling the time of ovulation with hCG or GnRH administered at estrus, to improve time of mating, has not yet yielded any significant improvements in farrowing rates or litter sizes. For single inseminations, it is not known whether synchronizing ovulation with hCG or GnRH will be practical and improve conception, farrowing rate, and litter size. Results with studies involving GnRH or hCG at the time of breeding are promising, however.

In cyclic females, the timing of estrus and ovulation is dependent on reproductive hormones, and effective manipulation could aid in the precision of reproductive management. The estrous cycle of the mature female occurs at regular 21-day intervals but can vary between 18 and 22 days. Occasionally, the cycle can extend as long as 24 to 26 days, as a result of an extended luteal phase. The mature sow or gilt normally expresses estrus for a 2-day period and ovulates between 35 and 45 hours after onset of estrus.[23] The estrous cycle can be divided into the luteal and follicular phases. The luteal phase of the estrous cycle begins from day 2 after onset of estrus when progesterone increases and lasts until about day 15, when progesterone declines. The follicular phase begins 5 to 6 days before estrus and is characterized by low progesterone and increasing estrogen.

LH initiates the process of final follicle maturation and ovulation, but concentrations during the cycle typically are low except for the ovulatory LH surge that occurs 40 hours preceding ovulation. LH is released in a pulsatile manner from the pituitary and has a 30- to 60-minute half-life in circulation. FSH is elevated during much of the cycle but declines at the time of luteolysis. This hormone has a half-life estimated to be about 2 hours and binds to follicles with multiple layers of granulosa cells. FSH is responsible for recruiting follicles to grow, from which only some will be selected for eventual ovulation.

Controlling reproduction may involve synchronization of estrus. Prostaglandin administration can be useful for shortening the length of the luteal phase and can reduce the interval to estrus by 3 to 4 days if given on days 12 to 13 of the estrous cycle. This is because prostaglandin receptors are low before day 12 and increase to maximal levels on days 13 to 16.[24] Prostaglandin typically is low in circulation throughout the estrous cycle except at the time of luteolysis in nonpregnant pigs. It is well established that in females lacking embryos and their estrogenic signal, prostaglandin of uterine origin is released from the uterus to destroy the functional lifespan of the corpus luteum.

By contrast, administration of hCG has been used to prolong the luteal phase. A dose of 1000 IU of hCG (Chorulon*) given on day 12 of the estrous cycle induced 63% to show an average cycle length of 33 days. Furthermore, the number of corpora lutea tended to be higher in treated gilts (mean, 15.2) than in controls (mean, 13.6). Similarly, estrogen can be used to lengthen the luteal phase. Normally, estrogen induces an LH surge and most of the physiologic and behavioral signs of estrus, including the standing response. Growing ovarian follicles are the source of circulating estrogen, which is low during the greater part of the estrous cycle but increases after luteolysis on day 14 and peaks 5 days later. During pregnancy, however, conceptus estrogen extends the life of the corpus luteum. A prolonged luteal phase also can be established by serial administration of estradiol between days 11 and 14 of the estrous cycle. Furthermore, estrogen given on days 12 to 13 can induce short pseudopregnancy (23–35 days), whereas administration between days 12 and 19 can induce long pseudopregnancy (greater than 50 days).[25] These extended corpora lutea are susceptible to exogenous prostaglandins. A preliminary study demonstrated that randomly cycling gilts can be rendered pseudopregnant by administration of estradiol implants left in place for 21 days. When injected with $PGF_{2\alpha}$ on the day of implant removal, five of six gilts were in estrus 5 to 7 days later.[26] This procedure is labor intensive, however, and estradiol is not yet approved for pigs.

Progesterone also can be used to synchronize estrus. Progesterone regulates the pattern of LH release and inhibits expression of estrus, even in the presence of estrogen. In the cyclic female, progesterone increases from baseline on day 2 of the cycle and begins a rapid increase to reach maximal concentrations between days 10 and 14 before declining. The first attempts at pharmacologic manipulation of the estrous cycle in cycling swine involved prolonged administration of progestogen to inhibit the final stages of follicular development. On withdrawal of progestogen, follicles matured rapidly and underwent ovulation within a predictable time interval. When 100 mg of progesterone in oil was injected daily into cycling gilts on days 15 to 28 of an estrous cycle, estrus was suppressed for the duration of the treatment period, and estrus followed the last progesterone injection within 6 to 7 days.

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In order to overcome the requirement for daily injections, several orally active progestational compounds have been studied for their utility in estrus control. One of the most successful compounds investigated for use in synchronizing estrus in cycling gilts was methallibure, which is a nonsteroidal compound that blocks gonadotropin release from the pituitary. When methallibure was fed for 19 days, fertile estrus was synchronously induced in a high proportion of animals within 5 to 8 days after the last feeding. Use of methallibure was halted and regulatory approvals were withdrawn in many countries, however, after reports of teratogenic effects. Alternatively, feeding 50 mg per day of 6-methyl-17α-hydroxyprogesterone acetate to gilts for 14 to 21 days resulted in induction of estrus in 50% of treated animals at 4 to 7 days after withdrawal, with 80% of treated animals ovulating. Estrus and follicular development were suppressed during treatment, and gilts were in fertile estrus an average of 4.4 days after drug removal.

Encouraging results eventually led to the development of the most successfully used orally active progestogen, allyltrenbolone (Regu-Mate*). When Regu-Mate was mixed with the daily ration or top-dressed onto the feed for 18 or 19 days at the rate of 10 to 15 mg/gilt/day, the average interval to estrus was 4.8 days, with normal ovulation and fertility at the time of synchronized estrus.[27] Although the 18-day treatment with Regu-Mate (15 mg/gilt/day) improved estrus synchronization when compared with 14-day treatment, no advantage of the 18-day treatment was found for subsequent farrowing responses.[28] The incidence of cystic follicles was low when compared with that observed with use of another orally active progestogen (6-chloro-Δ^6-17-acetoxyprogesterone) fed for 18 days. Regu-Mate also has been used to synchronize estrus for timed insemination without estrus detection. When gilts were artificially inseminated on days 5, 6, and 7 after Regu-Mate withdrawal, fertility and fecundity were equal to those in gilts that were tested for estrus and mated naturally 12 and 24 hours after the onset of estrus.[29] In practice, Regu-Mate, fed at the rate of 15 mg/gilt for 14 to 18 days, is an effective drug for synchronizing estrus in cycling swine. Treatment can be started on any day of the cycle. Underdosing Regu-Mate (less than 13 mg/day) may lead to increased incidence of cystic follicles, which could explain why some producers have reported variable responses in the field.[30] In the United States, Regu-Mate is approved for use only in horses, although it is used widely in swine throughout Europe.

A potentially useful approach to providing progestogen exposure for swine could be through a sustained-release implant. When randomly cycling gilts were administered an implant containing 6 mg of norgestomet, which was left in place for 18 days, five of six gilts were in estrus within 3 to 7 days after removal. Similarly, intravaginal placement of a progesterone-coated controlled internal drug release device for 14 days caused 17 of 24 (70.8%) gilts to come into estrus between 3 and 5 after removal.[31] At present, progesterone-releasing devices are not approved for use in swine in the United States.

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WEANED SOWS

Most sows return to estrus after average lactational periods of 16 to 22 days within 3 to 8 days. In these sows, estrus usually lasts 56 hours.[32] Although the time of ovulation is influenced by wean-to-estrus interval, ovulation typically occurs 41 hours after onset of estrus. Return to estrus after weaning in this time interval is important for optimizing breeding conditions and maintaining the integrity of farrowing groups. Unfortunately, not all sows return to estrus in this window or at all, and delayed returns are well recognized in primiparous sows in certain seasons of the year. Longer wean-to-estrus intervals have been reported in the seasons of spring, summer, and fall, with the latter two seasons mentioned more frequently in reports of seasonal infertility. Attempts at alleviating seasonal effects by altering photoperiod or lighting have had little impact, and standard lighting regimens appear to be adequate.[33] The effect of housing system also has been implicated as a causative factor in delayed return to estrus that even exposure to boars cannot overcome.

Group weaning is the most successful method for estrus synchronization in adult sows for facilitating production scheduling. The occurrence of post-weaning estrus, however, is influenced by many factors, including lactation length, parity, and season. Shortening lactation length is used to increase the both the number of litters per year and to reduce vertical disease transmission. When the litter is weaned after a 21-day lactation, follicular development typically resumes and estrus occurs within 4 to 7 days. When parity 1 sows have lactation lengths less than 14 days, however, they take an average of 10 days or more to resume cycling.[34] In fact, for all sows lactating for less than 12 days, the interval to return is 1.8 days longer than for sows lactating for more than 18 days.[35] Furthermore, early-weaning sows have reduced first-service conception rates (68% versus 87%) and fewer live embryos (10.4 versus 13) when compared with conventionally weaned sows.

The length of lactation appears to be important in the damped LH response to exogenous GnRH or estradiol during the first few weeks of lactation. In fact, the magnitude of the response improves dramatically as lactation progresses up to 3 weeks. This lactation response, however, is independent of physical nursing, litter size nursed, and the presence of circulating prolactin. In most sows after weaning, LH and FSH increase in concentration, but this increase is not entirely related to aspects of post-weaning estrus. Most sows that exhibit an early or normal return have increased LH pulse concentration, however. In lactating sows that express estrus in response to litter removal and exposure to boars, levels of FSH decrease and LH, estradiol, progesterone, and cortisol levels increase. Those sows that do not respond with estrus to these stimuli differ the most in FSH and estradiol concentrations. In conventionally weaned sows that express estrus, the peak of estradiol is observed 3 hours

before onset of estrus and 11 hours before the LH peak, at 40 and 30 hours before ovulation, respectively.[36] Prolactin characteristically is elevated in the lactating sow. Immediately after weaning, however, prolactin levels decline. Although thought to inhibit follicular activity during lactation, this hormone does not appear to be related to altered gonadotropin levels. Progesterone concentrations are low in sows before weaning, and baseline levels are maintained until they begin to increase about 30 hours after the LH surge. This again indicates that ovulation does not typically occur in sows during lactation, regardless of wean-to-estrus interval.

Reducing suckling intensity has been used to minimize the time interval from weaning to estrus after longer lactation periods. Wean-to-estrus interval is reduced by split-weaning the larger piglets from the nursing litter, 2 to 5 days before weaning. Reducing the wean-to-estrus interval also can be accomplished through partial weaning of the entire litter for 6 to 12 hours each day beginning at the second to third week of lactation. This technique has been effective in some but not all instances. The benefit of boar exposure during lactation is unclear, but boar contact after weaning increases LH and estrogen levels and generally hastens both return to estrus and the occurrence of ovulation. Continuous boar contact after weaning, however, may cause problems with heat detection, leading to a lower percentage of multiple matings.[37]

The ovarian status of the sow at weaning may be the key for understanding the reproductive performance of the sow after weaning. Ovulation does not usually occur with lactations lasting up to 28 days. At weaning and during lactation, ovaries contain small to medium-sized follicle populations. After weaning, follicles begin to grow and increase in size. Follicular growth in lactating and post-weaning sows is dependent on increasing LH and FSH. These hormones can be increased by substantial reduction in the intensity of suckling during lactation or by administration of exogenous gonadotropins. GnRH given in hourly doses has been shown to stimulate estrogen production and follicular development, resulting in estrus and ovulation in lactating sows after 2 to 3 weeks of lactation.

The wean-to-estrus interval is linked to sow body condition at weaning, feed intake in gestation and lactation, diet density, and environmental temperature. Despite all of these influences, it is clear that sows that lose less weight during lactation are in better body condition at weaning and subsequently rebreed sooner after weaning. It also is clear that inadequate protein and energy intake during lactation leads to excessive weight and backfat loss. One explanation for the delayed return to estrus associated with low nutrient intake is reduced follicular development and altered LH.[38] Support for this mechanism is the observation that administration of exogenous hormones to nutrient-deficient gilts, and to post-weaning sows in general, induces estrus and ovulation.[39,40] Feeding level after weaning appears to have little effect on interval from weaning to estrus but has been reported to be more beneficial for primiparous sows in the summer, when feed intake may be reduced by environmental temperatures. Energy intake is at least partly involved, because administration of exogenous insulin to sows at weaning at 0.4 IU/day for 4 days was found to initiate follicle growth and to improve return to estrus and litter size.[41]

Exogenous gonadotropins have been used to synchronize and improve post-weaning return to estrus, ovulation rate, and fertility in herds failing to show optimal post-weaning reproductive performance. Equine chorionic gonadotropin in combination with hCG has been used prophylactically to control the wean-to-estrus interval in sows. When PG600 was administered to sows at weaning, the percentage of sows returning in 7 days increased from 78% to greater than 90%, and the interval from weaning to estrus was reduced by 1 day. The numbers of pigs per mated sow is higher even though no overall increase in farrowing rate and litter size is observed. The increased number of pigs per mated sow results from the increase in number of mated sows that farrow. The lack of overall improvement in farrowing rate and litter size presumably results because time of ovulation after estrus is altered through a shortened wean-to-estrus interval.[40] A few reports, however, have identified a trend for improvement in overall litter size, even though farrowing rate was not improved. Investigators giving eCG following weaning improved litter size in both parity 1 and parity 2 sows but not in parity 3 or greater sows,[42] whereas 1200 IU of eCG tended to improve litter size (11.3 versus 9.3).[43]

Estrus and ovulation induction protocols use 750 to 1500 IU of eCG at weaning, followed by 500 to 1000 IU of hCG 72 to 96 hours later. Females inseminated within 24 hours after administration of hCG have greater than 90% conception rates.[44] Another ovulation induction protocol has been developed to deal with the adverse effects of early weaning in sows. At the time of farrowing, sows are reported to have developed follicles on their ovaries, which regress within 48 hours. Treatment at the time of farrowing with 1000 IU of hCG can induce these follicles to undergo ovulation, and the resultant corpora lutea progress through a normal maturation cycle, preventing a return to estrus for +/– 20 days, despite early weaning of the litter. Unfortunately, the reported success rate for this treatment was only 48%, so it has been deemed impractical for commercial herd application.[45]

Predicting or inducing the time of ovulation is paramount for obtaining satisfactory reproductive performance from an artificial insemination (AI) program. Use of real-time ultrasound scanning to image ovarian structures and dynamics has opened a new window of understanding on the ovarian outcome with various management and therapeutic practices. Successful single-service AI is a major goal of breeding management for both labor and material costs. This has been achieved in some herds simply by designing an AI protocol around the two thirds rule for the timing of ovulation after onset of estrus. It has been shown that natural ovulation occurs consistently at two thirds (67% to 71%) of the way through the estrual period, regardless of the duration of estrus.[46] It was further demonstrated that sows returning to estrus early after weaning (3 to 4 days) have delayed estrus-to-ovulation intervals when compared with those in sows returning to estrus at later intervals (after 4 days), and those returning to estrus even later (after 6 days) may

ovulate even sooner. One ultrasound study showed that in sows with early return to estrus after PG600 treatment at the time of weaning, estrus-to-ovulation interval was prolonged, such that 25% of treated sows did not receive an insemination within 24 hours of ovulation.[40] Another ultrasound examination in sows that failed to return to estrus within 7 days after weaning, and then were subsequently treated with PG600, showed a shortened estrus-to-ovulation interval (15.8 hours) compared with that in untreated controls (30.0 hours).[47] Some of these animals actually ovulated before expressing estrus, possibly in part because of the presence of medium-sized to large developing follicles induced to ovulate by the hormonal blend of hCG and eCG. Premature ovulation induced by hCG results in immature ova release, delayed insemination timing, and even inadequate uterine lining development for embryonic implantation, all of which foster a subfertile outcome. Recent information indicates that a localized uterine mechanism originating from boar seminal fluids during natural mating also may accelerate ovulation timing. It was subsequently shown that placement of seminal fluids or natural prostaglandins into the uterus may reduce the estrus-to-ovulation interval.

SUMMARY AND CONCLUSIONS

The indications for and methods of estrus synchronization in swine are varied, but all methods are based on controlling events leading to follicular maturation and ovulation or altering the luteal phase. The swine veterinarian plays a crucial role for assisting producers in making informed decisions about what, where, and how these pharmacologic and management-related interventions should be made to improve the productivity and efficiency of the modern swine farm. The normal hormonal changes involving reproduction must be thoroughly understood before exogenous hormones can be used properly. Hormones are not a substitute for improper management or poor pig flow. Heat stress (season), nutritional intake, and parity are three of the most common factors that can adversely affect wean-to-estrus interval and cause an increase in nonproductive sow days, which limits number of pigs weaned per sow per year. Gonadotropins have been used to successfully stimulate estrus and ovulation, thereby increasing the likelihood that swine producers can meet their mating targets.

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CHAPTER 101

Diagnosis of Pregnancy

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Early and accurate identification of pregnant and nonpregnant sows and gilts has a strong potential to increase reproductive efficiency of commercial swine farms. Detection of returns to estrus after mating, ultrasound techniques, and several other methods have been used for pregnancy diagnosis[1]; however, for various reasons, only three or four techniques currently are used in commercial farms.

An ideal pregnancy detection technique is not available, and most producers use either a combination of techniques or one technique that apparently fulfills their requirements. Some methods are restricted to research applications, and it is unlikely that these methods will be adapted for on-farm use. This chapter reviews the techniques for pregnancy diagnosis in swine; the practical applications, or lack thereof, are emphasized for each technique (Table 101-1). For most procedures, **sensitivity** (ability to detect pregnant animals, representing the proportion of pregnant animals that test positively) and **specificity** (ability to detect nonpregnant animals, representing the proportion of nonpregnant animals that test negatively) are used to assess accuracy.

DETECTION OF ESTRUS

A common pregnancy detection technique is observation of the sow for failure to return to estrus after mating. This technique is based on the premise that nonpregnant sows will return to estrus within 17 to 24 days after breeding. Detection of estrus is improved if the sow's behavior is observed in the presence of a boar.[2] Thus, this technique requires gestation facilities that are designed to allow daily fenceline contact between boars and sows, or the placement of the boar and sow in the same pen each day. Injections of estradiol or estradiol plus testosterone will enhance estrous behavior in sows that are not pregnant[3]; however, these steroids are not approved for use in swine in the United States.

An early study reported an accuracy of 39% for the detection of return to estrus between 19 and 25 days after mating.[4] By contrast, daily estrus detection throughout gestation enabled a 98% accuracy in predicting farrowing rate.[5] Albeit rarely seen, a false negative result can be obtained if a pregnant sow shows spontaneous estrus.[5] False positive results occur in the following scenarios:

- Sows become persistently anestrous as a result of cystic ovarian degeneration or inactive, acyclic ovaries, or become pseudopregnant.
- Submissive sows are housed in groups with dominant sows.
- Group sizes are too large to permit individual assessments of estrus.
- Sows are housed in stalls that do not permit the sow to detect the presence of the boar.
- The design of gestation facilities does not allow daily boar exposure to the bred sow.
- Breeding herd personnel fail to dedicate sufficient time to estrus detection.
- Breeding herd personnel lack adequate training to identify sows in estrus.

HORMONE CONCENTRATIONS

Serum concentrations of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), progesterone, and estrone sulfate were used as indicators of pregnancy. These hormone concentrations are dynamic, and considerable knowledge regarding endocrine changes in pregnant and nonpregnant sows is required for correct use of these techniques for pregnancy diagnosis. Owing to technical requirements, these methods are not used in the swine industry.

Prostaglandin $F_{2\alpha}$

The endometrium of the nongravid uterus secretes $PGF_{2\alpha}$ into the uterine vein between days 12 and 15 of the cycle, thereby inducing regression of the corpora lutea.[6] When viable embryos are present, $PGF_{2\alpha}$ is not secreted into the uterine vein.[7] The prostaglandin pregnancy test is based on the principle that if serum concentrations of $PGF_{2\alpha}$ are low (less than 200 pg/ml) or undetectable between days 13 and 15 after mating, the sow can be assumed to be pregnant.[8] Routine blood sample collection, without the use of indomethacin or other cyclo-oxygenase inhibitors, causes release of $PGF_{2\alpha}$ from blood constituents. This $PGF_{2\alpha}$ release leads to obvious problems with interpretation of serum $PGF_{2\alpha}$ concentrations; however, prostaglandin metabolites are more stable, and serum metabolite concentrations are reasonable indicators of prostaglandin concentrations.

The prostaglandin pregnancy test has approximately 90% sensitivity and 70% specificity. Accuracy is lower when animals have delayed returns to estrus, or when fetal death occurs and sows manifest pseudopregnancy. This diagnostic method can be used during early pregnancy, but its unreliability in the detection of nonpregnant animals and the necessity of extensive laboratory procedures limit its practical application.

Table **101-1**

Applications of Techniques Available for Pregnancy Diagnosis in Swine

	APPLICATION			TIME OF TEST DURING PREGNANCY*		
Technique	**Commercial**	**Diagnostic**	**Research**	**Ultra-early**	**Early**	**Late**
Early pregnancy factor			X	X		
Estrus detection	X				X	
Estrogen injections		X			X	
Laparoscopy			X		X	
Prostaglandin $F_{2\alpha}$			X	X		
Progesterone	X	X	X		X	
Estrone sulfate		X	X		X	
Rectal palpation		X				X
Vaginal biopsy		X	X		X	
Doppler ultrasonography	X	X				X
Amplitude-depth ultrasonography	X	X				X
Real-time ultrasonography	X	X	X		X	X

**Ultra-early:* before 18 days after breeding; *early:* 18 to 24 days after breeding; *late:* beyond 24 days after breeding.

Progesterone

Maintenance of the corpus luteum (CL) is the result of a blastocyst "signal" that is produced at 10 to 12 days after mating.[9] The blastocyst-induced maintenance of corpora lutea causes serum progesterone concentrations to remain high (greater than 5 ng/ml) throughout pregnancy. Thus, serum concentrations of progesterone are high in pregnant sows and gilts during the expected time of return to estrus and low (less than 5 ng/ml) in bred sows and gilts that fail to conceive.[10]

The interestrus interval for sows of various parities ranges from 17 to 24 days, with a mean of 20 to 21 days and a mode of 20 days.[11] Therefore, the optimal time to obtain blood samples for progesterone determinations is from 17 to 20 days if nonconceiving sows and gilts are to be identified before the time they return to estrus. The serum concentration of progesterone that most accurately discriminates the nonpregnant from the pregnant sow or gilt has not been determined. Concentrations of 4, 5, 7, 7.5, and 9 ng of progesterone/ml of serum were used to discern pregnancy status[5,10]; a concentration of 5 ng/ml is most commonly used.

The progesterone pregnancy test has greater than 97% sensitivity at 17 to 24 days, but specificity ranges from 60% to 90%.[5,12] False positive results are common when nonconceiving sows and gilts have delayed or irregular returns to estrus, and when nonpregnant sows and gilts are anestrous as a result of cystic ovarian disease.[5] False negative results may be the result of laboratory error because it is assumed that greater than 4 ng of progesterone/ml of serum is required for pregnancy maintenance in swine.[13]

Commercial enzyme-linked immunosorbent assays (ELISAs) to measure blood concentrations of progesterone in swine can be used on farms or in veterinary clinics, thereby reducing the need for laboratory-based radioimmunoassays. The necessity of collecting blood is a significant limitation of this method; however, methods to quantitate fecal progestins were evaluated for monitoring reproductive function in swine,[14] dogs,[15] and ruminants.[16] It was evident that the extraction and assay procedures are feasible alternatives to blood progesterone assays. Despite the potential use of fecal progestin determinations, direct applications of this methodology for pregnancy diagnosis have not gained popularity, nor have they been evaluated on a large scale in commercial pig production.

Estrone Sulfate

A high proportion of fetal estrogens is secreted from the uterus into the maternal circulation as estrone sulfate.[17] Estrone sulfate initially appears in the maternal circulation at approximately 16 to 20 days and rises linearly to peak levels between 25 and 30 days before decreasing to a nadir at 35 to 45 days.[18] A second increase in estrone sulfate occurs concomitantly with increases in other estrogens, commencing at 70 to 80 days and continuing until farrowing.

Urinary and serum estrone sulfate concentrations have been investigated for applicability as pregnancy tests.[5,19,20] A 10-fold to 100-fold increase in maternal estrone sulfate concentrations typically occurs between 25 and 30 days after coitus in sows that conceive.[21] Because estrone sulfate increases during early and late pregnancy, determination of estrone sulfate levels has potential applications as an early pregnancy diagnosis test and as a confirmatory test later during pregnancy. Parity, season, and day of pregnancy when blood was collected have some effect on estrone sulfate concentrations.

Serum estrone sulfate concentrations greater than 0.5 ng/ml are indicative of pregnancy, whereas concentrations less than 0.5 ng/ml are suggestive of nonpregnant status.[5,19] The estrone sulfate pregnancy test has greater than 97% sensitivity and greater than 88% specificity when serum samples are collected between 25 and 30 days of pregnancy.[5] False positive results may be attributable to a transient increase of estrone sulfate concentrations in some sows and gilts during proestrus.[18] False negative results were obtained in sows or gilts with a premature or delayed rise in estrone sulfate concentrations[22] or in sows and gilts that had less than 4 pigs in a litter.[5] As with other early tests of pregnancy, animals may be correctly diagnosed as pregnant but subsequently will fail to farrow if the fetuses die after the test has been conducted.

Pseudopregnant sows frequently retain the endocrine function of the corpora lutea for prolonged periods and may appear to be pregnant even though they no longer have viable fetuses. Other sows experience loss of their pregnancies, followed by development of acyclic, anovulatory ovaries or cystic ovaries, and similarly fail to show estrus for various time periods. Serum concentrations of estrone sulfate in pseudopregnant animals were similar to those in females that had not been mated and distinctly less than those in pregnant sows.[23]

Urinary concentrations of estrone conjugates also have been used to predict pregnancy and to diagnose fertility problems.[20] for these investigations, urine samples were obtained through the use of vaginal sponges. It should be noted that the glucuronide conjugate of estrone, rather than the sulfate form, was measured; thus, different quantitative procedures were required.

Quantitative commercial assay kits for the determination of estrone sulfate concentrations in serum from swine are not available. The need to collect blood (or urine) samples limits the practical application of this technique for pregnancy diagnosis in swine.

Early Pregnancy Factor

Early pregnancy factor (EPF) activity is dependent on the presence of two components: EPF-A and EPF-B. Factor A is formed in the uterus during estrus and pregnancy, whereas EPF-B is produced in the ovary and is associated only with pregnancy. The production of EPF-B is a result of a combined action of endocrine signals from the pituitary and the zygote.[24] Serum concentrations of EPF peak 24 to 48 hours after fertilization and exhibit a polyphasic pattern throughout gestation.[25]

Detection of EPF, based on a rosette inhibition test, is time-consuming and cumbersome and possesses other limitations. Various applications of EPF detection have been proposed for embryo transfer programs and assessment of infertility and early embryonic survival.[26] Several investigations indicated that EPF detection was a useful technique to evaluate human infertility and embryonic loss; however, little evidence is available to substantiate the use of EPF detection in commercial swine production. One study indicated that the rosette inhibition test was not quantitative or suitable for pregnancy diagnosis in swine.[27]

PHYSICAL METHODS

Radiography

Radiography is a seldom-used method for pregnancy diagnosis in swine. In research studies, it was used after the sixth week of pregnancy,[28] when the fetal skeleton begins to calcify. Fetal age, viability, and abnormalities were determined with radiography.[29] Equipment costs, potential health hazards to users, and the impracticality of radiography in production facilities render this technique unsuitable for pregnancy diagnosis in commercial swine.

Rectal Palpation

Pregnancy diagnosis by rectal palpation of the sow has been demonstrated to be practical and highly accurate.[30] Sows in the relevant study were examined while standing in gestation crates or pens, or while tethered. The technique involves examination of the cervix and uterus, together with palpation of the middle uterine artery to assess its size, degree of tone, and type of pulse. Before 21 days of gestation, rectal palpation had a 30% sensitivity, but the sensitivity increased to 75%, 94%, and 100% in animals at 21 to 27, 28 to 30, and 60 days to term, respectively.[30]

The pelvic canal and rectum often were too small for the procedure to be used on low-parity sows. False positive results were obtained if the external iliac artery or one of its branches was mistakenly identified as the middle uterine artery. False negative results, presumably due to errors in palpation technique or performance of palpation too early, were more common than false positive diagnoses. Despite the potential application of this technique, it has not gained popularity in North America.

Laparoscopy

Initially, laparoscopy was adapted in swine for studies of ovarian activity,[31] and a research study revealed that pregnancy can be diagnosed with 100% accuracy using this technique.[32] Differences in color were noted between gravid and nongravid uteri, because of the greater blood flow to the gravid uterus and increased uterine tone in response to hormonal stimulation. Observation of the corpora lutea allowed an estimation of the number of ova shed, and follicular development in accompaniment with regressing corpora lutea was apparent in animals returning to estrus. When this test was conducted between 16 and 20 days after coitus, pregnancy could be diagnosed before return to estrus in nonconceiving animals. Obviously, laparoscopy is limited to research applications.

Vaginal Biopsy

Histologic changes in the vaginal mucosa characterize specific stages of the estrous cycle and pregnancy.[33,34] This relationship was the basis for a study in which vaginal biopsies were used to diagnose pregnancy in swine.

Specimens of vaginal epithelium, obtained with a biopsy instrument, were prepared for histologic examination. Histologic sections of epithelium were categorized according to the number, types, and arrangement of superficial epithelial cells.[35] This procedure can be conducted between 18 and 22 days after mating; however, the most reliable results are obtained when the specimen is collected after 25 days of gestation.[36] Erroneous test results were obtained for sows and gilts with irregular returns to estrus, sows and gilts affected with cystic ovarian degeneration, immature gilts, anestrous sows, and sows in which resorption of fetuses occurred after pregnancy testing. False negative results were noted when specimens were taken in late pregnancy.[36] The vaginal biopsy technique is impractical, and the potential delay in diagnosis with laboratory procedures reduces the usefulness of this technique for routine pregnancy detection.

ULTRASOUND TECHNIQUES

Mechanical ultrasound devices commonly are used because they are easy to operate and commercially available and are perceived as being accurate. Three types of ultrasound units are available for pregnancy diagnosis in swine. Each instrument functions on a different scientific principle.

Doppler Ultrasonography

Doppler instruments utilize the transmission to and reflection of ultrasound beams from moving objects such as the fetal heart and pulsating umbilical vessels or uterine arteries.[37] Blood flow to the uterine artery in the pregnant sow and gilt is detected as a regular 50 to 100 beats per minute, whereas blood flow in the umbilical arteries is detected at 150 to 250 beats per minute.[38]

Two types of transducer probes—namely, an abdominal and a rectal probe—currently are available for use with the Doppler instruments. The abdominal probe is positioned on the flank of the animal, lateral to the nipples, and aimed at the sow's pelvis area. The ultrasound waves are emitted and received by transducers and are converted to an audible signal. The rectal probe functions similarly, with the obvious exception of the positioning of the transducer. One study found no differences in sensitivity and specificity between the rectal and the abdominal probes.[5] Other reports showed that sensitivity was greater than 85% and specificity was greater than 95%.[39,40] Optimal results were obtained when pregnancy determinations were made later than 29 to 34 days; sensitivity decreased with scanning performed earlier in gestation.[40]

Doppler ultrasound techniques had greater sensitivity, specificity, and overall accuracy than amplitude-depth ultrasonography,[40] but substantial variation in the accuracy of different models of amplitude-depth devices may make the results of such comparisons misleading.[5,41] After 1 month of gestation, the Doppler devices apparently had greater specificity, whereas the amplitude-depth machines had greater sensitivity.[40]

The likelihood of false positive results was increased if examinations were done when sows and gilts were in proestrus or estrus, or if sows and gilts had active endometritis.[5] False negative results were obtained if examinations were conducted before 30 days, if examinations were conducted in a noisy environment, or if feces became packed around the rectal probe.[5,40] Another disadvantage associated with use of Doppler techniques is that training is required in the use of the instrument.

Amplitude-Depth (A-Mode or Pulse Echo) Ultrasonography

Amplitude-depth machines utilize ultrasound waves to detect the fluid-filled uterus.[42] A transducer is placed against the flank and oriented toward the uterus. Because the contents of the gravid uterus differ in acoustic impedance from that of adjacent tissues, some of the emitted energy is reflected to the transducer and is converted to an audible signal, a deflection on an oscilloscope screen, or illumination of a light (diode) or series of lights.[42]

In one study, pregnancies were not confirmed before 20 days, but progressive improvement in pregnancy detection was observed from day 20 until day 30.[41] From approximately 30 days until 75 days after breeding, the overall accuracy in the determination of pregnancy commonly was greater than 95%.[40,43] The percentage of false negative and uncertain determinations increased from 75 days until farrowing.[41] These changes in accuracy parallel alterations in volume of allantoic fluids and fetal growth.[44]

The amplitude-depth instruments had greater sensitivity but less specificity compared with Doppler instruments.[40] Some models of amplitude-depth instruments, however, were more severely affected by low sensitivity and specificity.[5] Errors in the placement of the transducer resulted in the detection of a fluid-filled urinary bladder, which yielded a falsely positive diagnosis of pregnancy.[40] False positive results were obtained when sows were affected with endometrial edema caused by zearalenone toxicosis or pyometra, or when the litter died and was neither aborted nor resorbed.[5] False negative results were noted when the test was performed before 28 days of gestation or after day 80.[41]

Real-Time Ultrasonography

Real-time ultrasound scanners have been used to evaluate the reproductive tracts of mares,[45] heifers,[46] and bitches[47] and for pregnancy diagnosis in sows and gilts.[48,49] Also, domestic animals such as sheep[50,51] and goats[52] have been scanned for pregnancy diagnosis and reproductive problems.

Real-time ultrasound techniques use beams of sound waves emitted from a multi-transducer. These beams travel in straight lines through different tissues until they encounter boundaries of differing acoustic density. Then they are reflected back to the same transducer and converted to electrical signals, ultimately being displayed on a monitor as a two-dimensional cross section of the interior of the animal.[48] The various tissues reflect the sound waves at differing amounts based on their acoustic

density. Accordingly, tissues such as bone reflect a large portion of the emitted waves and appear white on the screen, whereas other tissues are various shades of gray. Fluid-filled structures such as the urinary bladder or amniotic vesicles are nonechogenic and appear black because they do not reflect any sound waves.[50] The combination of these reflections generates the overall image viewed on the screen.

Previous work with real-time scanning utilized abdominal probes, with the transducer placed against the flank of the animal. This positioning is lateral to the nipples and posterior to the navel, similar to that for other pregnancy detection devices being used currently.[48] From this position the probe is directed toward the back of the animal, allowing the ultrasound waves to pass through the uterus before returning back to the transducer. Amniotic vesicles became visible on real-time scans around 18 or 19 days after breeding; embryos were observed by 21 days and easily detected by 25 to 32 days.[53] Fetal movement was observed after day 60.[48] Recently, 5.0- and 7.5-mHz probes were used transrectally to determine the pregnancy and estrus status of breeding females.[54] Use of the transrectal probes may be marginally more time-consuming than use of the abdominal probes; however, the increased structural detail of the ovaries and early pregnancy (days 16 to 20) improves the usefulness of transrectal ultrasonography for use in female pigs. General characteristics of real-time ultrasound probes and representative images are provided in Table 101-2 and Figure 101-1, respectively.

Table **101-2**

General Characteristics of Probes for Real-Time Ultrasonography

Probe	Depth of Field (cm)	Resolution	Timing*
3.5 mHz	10–12	Low	Days 25–26
5.0 mHz	7–10	Medium	Day 23
7.5 mHz	5–7	High	Day 21

*Day(s) of gestation on which pregnancy can be diagnosed with reasonable reliability.

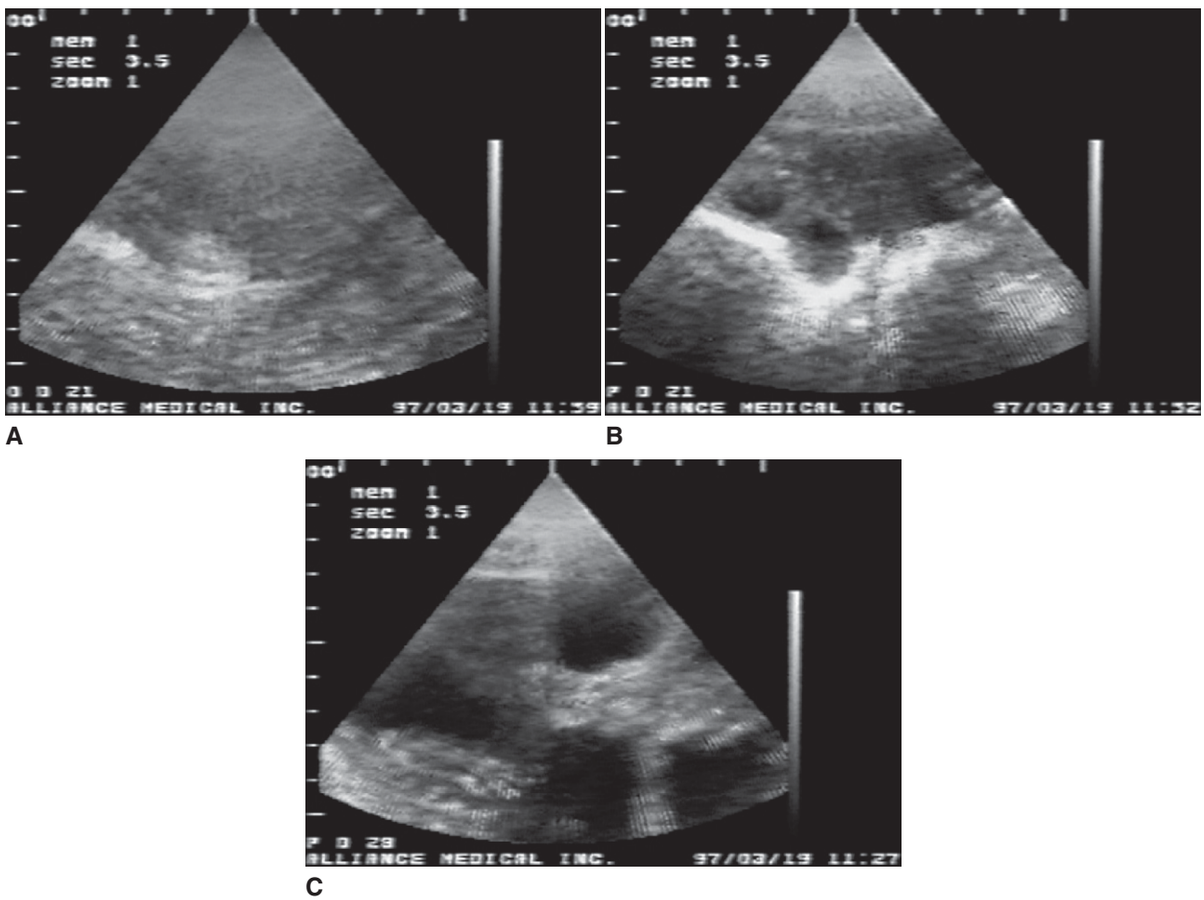

Fig. 101-1 Real-time ultrasonographic images of sow uteri observed with a 3.5-mHz sector probe. **A,** Image from an open sow at day 21 of the estrous cycle. **B,** A day 21 pregnancy. **C,** A day 28 pregnancy.

Early research indicated that real-time ultrasound scanning had greater than 95% sensitivity and specificity at 22 days of gestation or later.[48] False negative results were obtained if the scans were performed before day 22 of gestation, when the vesicles were too small to be detected regularly. False positive results were obtained in sows and gilts that had cystic ovarian degeneration or uterine infections resulting in accumulations of fluid.

Research with ewes showed that pregnancy status of the animal was a major determinant in the time needed to make a diagnosis using real-time ultrasound scanning. Pregnant ewes were diagnosed in about 10 seconds, whereas scanning of nonpregnant animals took much longer.[51] In general, similar time is required for real-time ultrasound scanning for pregnancy diagnosis in female pigs.

Our studies demonstrated several sources of variation affecting the success of real-time ultrasound evaluation for pregnancy diagnosis.[55] Sources of variation include type of probe, day of gestation when scanning is initiated, and technician skill (Tables 101-3 and 101-4). Parity is a potential source of variation that has not been thoroughly examined. With the exception of the field trials, most controlled studies were conducted with gilts. It is possible that scanning procedures need to be adjusted for the increased abdominal size and the changes in anatomic location and size of the reproductive tract in multiparous sows.

Because the initial purchase price ($7000 to $15000) of a real-time ultrasound instrument is considerably higher than that of other pregnancy detection devices, producers and veterinarians often are reluctant to purchase a real-time ultrasound unit. For farms with 100 or 200 sows, this reluctance may be justified. With larger herd sizes, however, the additional costs of a real-time ultrasound instrument quickly diminish on a per sow basis (Table 101-5).

Factors affecting the benefits of real-time ultrasonography are interrelated and can have compounding effects on the number of open sow days saved and the money saved per sow. The application of real-time ultrasound scanning as a pregnancy detection technique has greater impact when the farrowing rate is low. The percentage improvement, which is defined as percent of open sows detected by real-time ultrasound evaluation at 23 days that were missed by traditional detection methods (e.g., A-mode, Doppler) at 28 days or sooner, also has dramatic effects on the benefits of real-time ultrasonography. Addi-

Table **101-3**

Technician Skill and Success of Real-Time Ultrasonography for Pregnancy Diagnosis in Swine*

	3.5-mHz SECTOR PROBE		5-mHz LINEAR PROBE	
Indicator	**Tech A**	**Tech B**	**Tech A**	**Tech B**
Sensitivity (%)	84.6	68	97.3	91.1
Specificity (%)	95.7	88	91	85.4
Accuracy (%)	89.9	78	94.9	88.2

*Performed at 21 days after mating.

Table **101-5**

Costs of Real-Time Ultrasonography for Weekly Testing in Swine Breeding Herds of Different Sizes

No. of Sows Tested/Week	Total No. of Sows*	ADDITIONAL COSTS†	
		$/Sow Tested	$/Year
25	500	2.214	2,878
50	1,000	1.072	2,787
100	2,000	0.501	2,605
200	4,000	0.215	2,241
300	6,000	0.120	1,877
400	8,000	0.073	1,513
500	10,000	0.044	1,149
600	12,000	0.025	785
700	14,000	0.012	421
800	18,000	0.001	57

*Hypothetical herd size.
†Costs are based on a 3-year life of the real-time ultrasound unit, an initial price of US $7000 for the unit, and adjustments for labor savings (one real-time ultrasound assessment versus two scans with an A-mode instrument). The annual costs of the real-time ultrasound unit include repairs, supplies, and amortization at 8% of the annual percentage rate (APR).
Data from Armstrong JD, Almond GW, White S, et al: Accuracy and economics of RTU pregnancy detection, and comparisons with A-mode. North Carolina State University. Available at: http://mark.asci.ncsu.edu/Reproduction/rtu/armstrong.htm (accessed 1997).

Table **101-4**

Day of Gestation and Success of Real-Time Ultrasonography for Pregnancy Diagnosis in Swine

	DAY OF GESTATION*				
Indicator	**17–20**	**21–23**	**24–30**	**38–44**	**52–58**
Sensitivity (%)	78.7	100	99.6	100	100
Specificity (%)	50	58.3	70	44.1	36.4
Accuracy (%)	74.5	97	97.8	98	98.7

*A 5-mHz sector probe was used in this field trial.

Table **101-6**

Production Benefits of Real-Time Ultrasound Scanning by Sow Numbers Scanned and Day of Gestation*

	NPSD/YEAR SAVED BY DAY OF GESTATION		
Number of Sows	**23**	**25**	**27**
500	570	526	482
1,000	1,143	1,055	967
2,000	2,287	2,112	1,936
4,000	4,576	4,224	3,873
6,000	6,864	6,337	5,809
8,000	9,152	8,449	7,746
10,000	11,440	10,561	9,682

*I.e., number of nonproductive sow days (NPSD)/year saved, as affected by number of sows and day of gestation on which scanning is initiated.
Assumptions: 1% improvement above results with A-mode ultrasonography and an 85% farrowing rate.
Data from Armstrong JD, Almond GW, White S, et al: Accuracy and economics of RTU pregnancy detection, and comparisons with A-mode. North Carolina State University. Available at: http://mark.asci.ncsu.edu/Reproduction/rtu/armstrong.htm (accessed 1997).

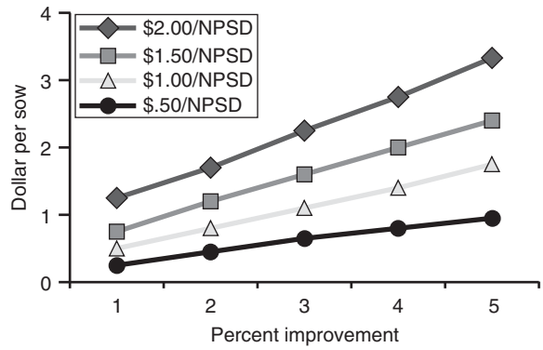

Fig. 101-2 Money saved per sow is influenced by both the percentage improvement and the cost of a nonproductive sow day (NPSD). The real-time ultrasound scanner was used at 23 days after mating, and the data are based on scanning 200 sows per week, with a 75% farrowing rate. The percentage improvement is based on a comparison of real-time ultrasound scanning at 23 days with amplitude-depth scanning at 28 and 35 days of gestation.

tional savings are gained when producers are willing to institute a 100% culling policy, rather than a standard culling policy (cull after the second return to estrus). Open sows are culled immediately after pregnancy detection with the 100% culling policy.

We have observed that many producers become very proficient with the use of real-time ultrasound techniques. These producers can diagnose pregnancy as early as day 23 (sometimes earlier). This proficiency is useful in assessing pregnancy status in breeding groups, when the test day includes animals at days 23 to 28 of gestation. It is not surprising that the greater benefits are gained with the use of real-time ultrasound scanning in large herds or as more sows are tested (Table 101-6). Again, the greatest benefits are gained when early testing is implemented in herds with low farrowing rates. The cost of an open day or nonproductive sow day (NPSD) varies from farm to farm. As the cost of an NPSD increases, the savings per sow with use of real-time ultrasonography also increase (Fig. 101-2). It also is evident that if real-time ultrasound scanning detects a greater percentage of open sows than is found with the traditional methods, then additional savings per sow will result.

Real-time ultrasound scanning appears to be more versatile than the other methods of pregnancy diagnosis currently available. Besides pregnancy detection, it has been used to assess reproductive disorders in goats[52] and to evaluate carcass characteristics in growing pigs.[56] These findings lend further support to the potential for other uses for this technique in swine production. For instance, pseudopregnant sows and gilts with uteri containing mummified fetuses have been differentiated from pregnant sows.[57] Recently, it was demonstrated that real-time ultrasound scanning is an effective technique to identify pseudopregnant sows between days 65 and 75 after mating.[58] Although the uterus of a pseudopregnant sow may contain fluid, the failure to observe fetal skeletons is indicative of pseudopregnancy. Furthermore, past research showed that this technique was useful to identify and distinguish sows and gilts with endometritis from females in later stages of pregnancy.[57]

SUMMARY AND CONCLUSIONS

The advantages of sensitive and specific methods for early pregnancy diagnosis in swine include early detection of conception failure, forecasting production levels, and early identification of nonpregnant animals, which facilitates decisions regarding culling, treatment, or rebreeding. At present, detection of nonconceiving sows that return to estrus and use of amplitude-depth ultrasonography are the most widely used methods of pregnancy diagnosis. Despite routine use of these traditional methods, a common finding is that many sows either fail to farrow after being considered pregnant or return to estrus at irregular times during a presumed pregnancy.

Perhaps the most promising technique for porcine pregnancy detection is real-time ultrasound scanning. This method is used routinely in a variety of species; however, applied research and improved technology have enhanced its applications in commercial sow farms. Perhaps the most intriguing aspect of real-time ultrasound scanning is that the user can visualize the uterus and its contents. Despite the obvious need for improved pregnancy diagnosis abilities for swine producers, little progress or changes were made until the development and application of real-time ultrasound techniques.

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CHAPTER 102

Infertility Associated with Abnormalities of the Estrous Cycle and the Ovaries

GLEN W. ALMOND

Various reports have demonstrated the role of infectious agents in porcine reproductive failure. For most infectious agents, researchers and veterinary practitioners have elucidated the pathogenesis, modes of transmission, and reasonably effective control programs. By contrast, porcine reproductive and respiratory syndrome virus (PRRSV) disease continues to be difficult to control, and the swine industry recognizes the economic impact of the virus on all phases of production. PRRSV disease continues to be detrimental to reproductive performance in sow herds, with variable severity of clinical signs in affected animals. With these widespread problems associated with PRRSV, pig producers often attribute reproductive failure to the virus when in fact the underlying causes are noninfectious. Unfortunately, the precise pathogenic mechanisms of noninfectious causes of reproductive failure also are elusive, and the interactions between the various factors affecting reproduction create major challenges to any efforts to improve the performance of the breeding herd.

Various investigators have examined the physiologic control of estrus, ovulation, conception, and pregnancy; however, the endocrine changes associated with these processes are more complex than was previously believed. Season, nutrition, environment, and management are well recognized for their potential to alter reproductive processes. In addition, recent studies revealed that various components of the immune system also possess profound roles in porcine reproduction. This chapter provides information regarding the physiologic mechanisms and "management" factors involved with infertility due to abnormalities of the porcine estrous cycle.

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Various investigators have examined the physiologic control of estrus, ovulation, conception, and pregnancy; however, the endocrine changes associated with these processes are more complex than was previously believed. Season, nutrition, environment, and management are well recognized for their potential to alter reproductive processes. In addition, recent studies revealed that various components of the immune system also possess profound roles in porcine reproduction. This chapter provides information regarding the physiologic mechanisms and "management" factors involved with infertility due to abnormalities of the porcine estrous cycle.

PROLONGED WEANING-TO-ESTRUS INTERVAL

Most pig producers attempt to have 95% (or more) of sows bred within 7 days after weaning (Fig. 102-1). Producers strive to achieve this goal to minimize the number of nonproductive sow days (NPSDs). An NPSD is a day on which a sow is not pregnant or lactating, and the number of NPSDs is recognized as an economic loss to the producer. The ability to have the vast majority of weaned sows bred within a week is helpful to maintain the integrity of breeding groups and provides a natural synchronization of estrus for breeding programs. In addition, the results of several studies indicate that the fertility, as measured by farrowing rate and litter size, is less in sows returning to estrus after 7 days than in sows that returned to estrus within 4 to 6 after weaning. It was suggested that animals returning to estrus 7 to 10 days after weaning are "subfertile."[1] Under certain circumstances, it may be warranted to postpone breeding these animals until the second postweaning estrus (i.e., skip a heat).

Culling surveys historically reported that 5% to 30% of sows are culled for failing to return to estrus after weaning. Many of these sows remained anestrous for prolonged periods (greater than 30 days), and their ovaries were acyclic. Such acyclic ovaries have small follicles (less than 5 mm in diameter) and well-regressed corpora lutea. At present, the incidence of these chronically anestrous sows appears to be less than that of sows with a relatively brief delay in return to estrus after weaning. For both types of anestrus, the condition is most prevalent in primiparous sows during the summer months.

Factors affecting the sow's ability to return to estrus after mating have been were reviewed.[2] The onset of estrus may be hastened or delayed, depending on the quality of management in both the breeding and the farrowing facilities. For example, sows consuming insufficient feed or feed with suboptimal energy or protein content during lactation in the summer months have longer WEI than sows with optimal feed and nutrient intake. Evidently, diminished feed intake during the first week of lactation, the last week of lactation, or throughout lactation contributes to postweaning reproductive failure.[3] It is apparent that the greatest challenge is to ensure sufficient feed intake by primiparous sows (Fig. 102-2). To overcome the problem of suboptimal energy intake, fat (5% of the diet) is added to feed to increase caloric intake. The protein and amino acid requirements of highly prolific sows also may require adjustments, particularly primiparous sows nursing large litters of piglets. In regard to daily feed intake, feeding schedules for lactating sows typically are adjusted to provide fresh feed at least three times a day. Furthermore, evaporative cooling is used in the farrowing rooms to improve feed intake during the summer months.

Various weaning strategies, based on the removal of suckling-induced inhibition of follicular growth, have been used to shorten the WEI. Weaning the heaviest half of a litter 2 days early shortened WEI, and when this split weaning was initiated early enough (at 5 days), the WEI was reduced to 1 to 2 days.[4] Potential benefits of this technique are to shorten WEI and to create a more synchronous return to estrus. The increased synchrony of estrus facilitates breeding and artificial insemination (AI) programs. Litter-sow separation (altered suckling) for 6 to 12 hours each day shortened the WEI if the previous WEI was longer than 6 days. This technique is labor intensive and provides limited advantages to pork producers.

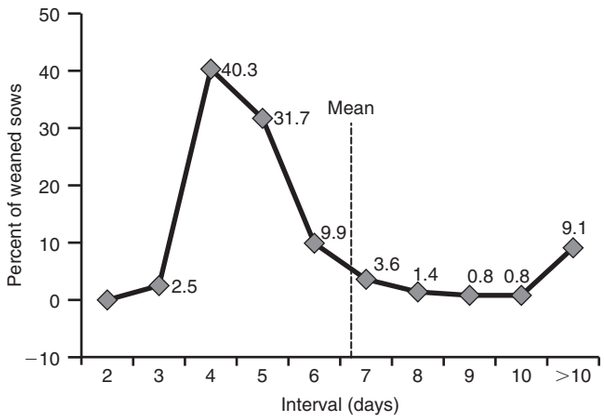

Fig. 102-1 Percent of sows (n = 2530 observations) returning to estrus from 2 to later than 10 days after weaning in a commercial farm. Although a majority of sows returned to estrus within 7 days after weaning, the mean weaning-to-estrus interval (WEI) was 6.77 ± 7.7 days. Obviously, a minority of the animals were not detected in estrus for extended periods of time after weaning. The median, which is a useful indicator of the population WEI, was 5 days. Collectively, the WEI accounted for 17,137 nonproductive sow days.

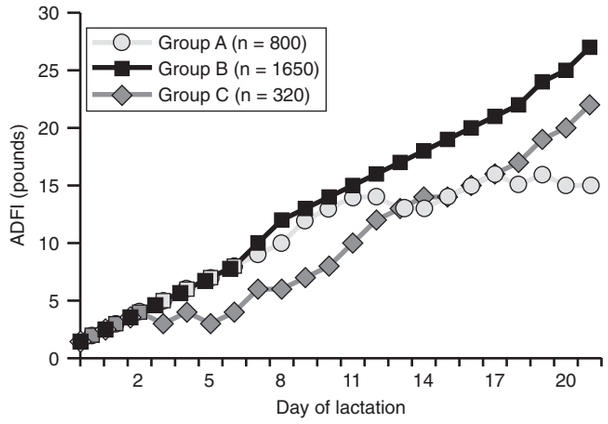

Fig. 102-2 Average daily feed intake (ADFI) in three groups of sows in a commercial farm. Feed intake increased steadily during lactation in multiparous sows (Group B) without postpartum health problems. Despite increasing feed allowance, feed intake reached a plateau of approximately 15 pounds per day in primiparous sows (Group A). Sows (Group C) with postpartum health problems and diminished ADFI in the first week of lactation failed to match the ADFI achieved in healthy animals. Groups A and C represent "high-risk" animals and are more likely to have prolonged weaning-to-estrus intervals.

A previous study indicated that the WEI is inversely related to lactation length.[5] This correlation generally is accepted; however, females of most genetic lines and parities can be weaned at 14 days or earlier and return to estrus within 7 days.[6] The most extreme example of short lactation length is the "zero-weaned" sow. Piglets are removed from these sows at birth. One investigation demonstrated that many of these animals will return to estrus at 14.8 ± 0.5 days after birth/weaning.[7] Although lactation length has the potential to lengthen WEI, most modern sow farms routinely wean piglets from sows at less than 21 days. The possible negative influence of short lactation length must be assessed for each farm (Fig. 102-3) and compared with the potential economic benefits of the early-weaning sow.

Numerous exogenous hormone preparations have been assessed for inducing estrus in the postweaning sow; however, an equine-human chorionic gonadotropin (eCG/hCG) combination, PG600, has received the greatest attention. It was suggested that the administration of PG600 to sows at weaning reduced the WEI and the incidence of anestrus in post-weaning sows, if a problem existed on the farm.[8] Subsequently, it was revealed that PG600 treatment of postweaning sows reduced the WEI from 7.8 ± 0.6 days to 6.0 days and from 6.4 ± 0.7 to 4.7 days in first- and second-parity sows, respectively, during the summer months.[9] These studies indicated that potential benefits could be obtained by using PG600 in postweaning sows, particularly in the summer. It is essential to conduct on-farm trials with any exogenous hormone combination to demonstrate its cost-effectiveness.

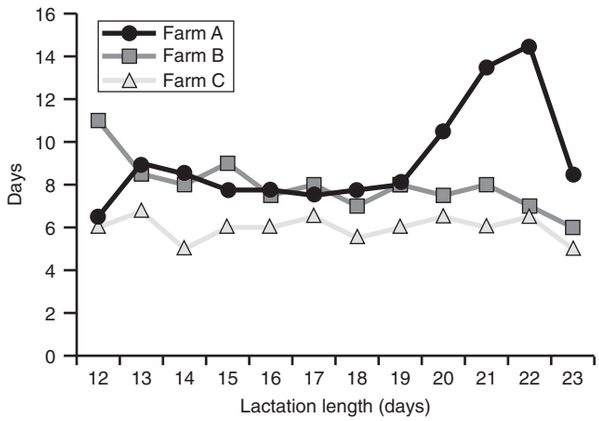

Fig. 102-3 The influence of lactation length on weaning-to-conception interval (WCI); days) for three 1200-sow farms (farm A, ●; farm B, □; farm C, ▲). The interval for each lactation day represents the mean of 100 to 400 observations. Most animals returned to estrus and were mated within 7 days of weaning. It was evident that lactation length had minimal effects on weaning-to-estrus interval (WEI) and WCI in farm C. By contrast, sows with a lactation length greater than 20 days had extended WEI and WCI in farm A. Sows with a lactation length less than 12 days tended to have a WEI greater than 7 days, with considerable sow-to-sow variation *(data not shown)* in the three farms.

The most common problem with the control of estrus and ovulation in the postweaning sow is the inability of personnel to detect the onset of estrus. Without appropriate boar exposure, it is extremely difficult or impossible to adequately assess estrus in the pig. Recently, it was demonstrated that 10 minutes of daily contact between postweaning sows and mature boars reduced the WEI. In fact, 10 minutes of daily contact between postweaning sows and estrogenized "estrous" females reduced the WEI in a fashion similar to that for sow-boar contact.[10] Obviously, estrus control requires certain behavioral and physical cues between animals.

FAILURE TO DETECT OVULATING FEMALES ("SILENT HEAT")

In approximately 20% (or more) of ovulating gilts and sows, signs of estrus are not detected by farm personnel observing the animals for estrus. Obviously, suboptimal estrus detection techniques contribute to the failure to observe estrus. The failure to use a mature boar for estrus detection is a common problem, particularly when a female exhibits subtle signs. With the increased use of artificial insemination (AI), some producers find effective estrus detection to be one of the main challenges associated with the transition from natural mating programs to AI programs.

It is easy to blame breeding herd personnel for deficiencies in estrus detection; however, it is not uncommon for females to have a propensity for reduced intensity of estrous signs. To distinguish problems with estrus detection from physiologic or pathologic causes of anestrus, examination of reproductive tracts at slaughter will reveal the presence of acyclic or functional ovaries. If a majority of animals have acyclic ovaries at slaughter, then the clinical assessment is directed at finding the causes of the acyclicity. Conversely, if most animals have active ovaries, as indicated by the presence of corpora lutea or regressing corpora lutea with developing follicles, it is apparent that estrus was not detected in the animals, and questionable estrus detection methods must be considered. Another technique to confirm acyclicity in female pigs is to collect two blood samples approximately 10 days apart. Failure of the serum progesterone concentration to reach 4 ng/ml or greater in either sample is indicative of ovarian acyclicity. At present, a rapid and inexpensive test (Target) is commercially available for on-farm assessment of serum progesterone concentrations.

It is evident that social environment contributes to the failure to detect estrus. Inadequate or inappropriate boar exposure and continuous boar exposure reduce the likelihood of detecting estrus by this means. Group size (8 animals or less is optimal) and space allowance (an area of 2 m^2/gilt is optimal) also influence estrus detection.

Veterinarians typically are asked the basic question "Can sows or gilts have a silent heat?" Previous research results indicated that silent heat or estrus is physiologically possible.[11] Sufficient estradiol was produced to induce an LH surge and subsequent ovulation, yet these animals failed to exhibit signs of estrus, including

reddening and swelling of the vulva. Evidently, the critical threshold of estradiol concentrations to induce ovulation differs from the threshold necessary for estrous behavior and vulvar changes. Despite this presumptive physiologic phenomenon, clinical experience typically confirms that "silent heat" often is an excuse for poor estrus detection.

CYSTIC OVARIAN DEGENERATION

Slaughterhouse surveys from the 1970s and 1980s reported that approximately 5% to 10% of sows culled for infertility are affected with cystic ovarian degeneration (COD). With the intensification of pig production, routine use of confinement facilities, and high replacement rates (greater than 50% per year), it would not be surprising to observe an increased incidence of COD in female pigs in the North American pig industry. Unfortunately, large-scale surveys of culled female pigs have not been conducted in recent years, so the precise incidence of COD is speculative.

Multiple large, multiple small, and single cysts occur in the ovaries of sows; however, the behavior and physiologic events differ between animals affected with each type of cyst. Most of the multiple large cysts have some luteinized tissue and produce sufficient progesterone to inhibit estrous cyclicity. Some sows fail to exhibit estrus and are culled from the herd. By contrast, other sows have irregular estrous cycles or exhibit "nymphomania." Typically, these sows have multiple small or follicular cysts on the ovaries. Because these animals fail to ovulate, it is unlikely that they conceive. Single ovarian cysts rarely affect fertility or the estrous cycle of sows; their presence is noted in occasional sows at slaughter. Because ovarian cysts do not regress with or without hormone intervention, it was postulated that the single ovarian cysts may represent the first step in a progressive process of degeneration affecting multiple follicles.

It is not unusual to observe corpora lutea with fluid-filled cavities. These particular corpora lutea commonly are referred to as cystic corpora lutea and presumably do not interfere with fertility of female pigs. The precise pathogenesis of the cystic corpora lutea is unknown; however, corpora lutea of normal size with fluid-filled cavities are relatively common in diestrous sows. One study demonstrated that luteal cells are physiologically similar in corpora lutea with cavities and in "solid" corpora lutea.[12]

Results of a recent study revealed that tonic and pulsatile patterns of luteinizing hormone (LH) release in sows with cystic ovaries are comparable with those in diestrous sows.[13] Evidently, the presence of ovarian cysts does not inhibit the hypothalamo-hypophyseal axis. Unfortunately, affected sows produce insufficient LH to complete luteinization of the cysts, or the luteolytic mechanisms are inadequate to promote regression.[14]

It is evident that the diagnosis of cystic ovaries poses a difficult challenge. Because serum concentrations of progesterone, estradiol, LH, and cortisol are similar in sows affected with COD and in diestrous sows,[13] the determination of serum hormone concentrations offers limited diagnostic value. It is not unusual to observe cystic ovaries during real-time ultrasound evaluation for pregnancy diagnosis or during scanning procedures to monitor follicle growth. Real-time ultrasonography offers a promising technique to assess COD in female pigs; however, the required time, training, and expense limit the practical application of this modality for evaluation for COD on commercial farms. Consequently, producers rely on estrus detection methods and culling policies to minimize the influence of sows with COD on nonproductive sow days and the reproductive performance of the breeding herd.

PSEUDOPREGNANCY AND NOT-IN-PIG SOWS

In general, two classifications of pseudopregnancy in sows are recognized. Not-in-pig (NIP) sows were bred, failed to exhibit an obvious return to estrus, were diagnosed pregnant, yet failed to farrow any pigs. This NIP status represents the extreme version of pseudopregnancy. The second classification of pseudopregnancy involves sows that were bred, failed to exhibit estrus at 21 days after mating, and finally show estrus at 30 to 35 days or >45 days after mating. Thus, pseudopregnancy can be classified as long term or short term.

European and Canadian culling surveys indicated that 3% to 20% of culled sows were classified as NIP at the expected time of farrowing.[15,16] An increased number of NIP sows is more commonly observed in the November-to-January period than at other times of the year—these sows were bred during the hot summer months. The overall incidence of NIP sows typically is 1% to 3% of all bred animals. If the incidence exceeds 3%, then interventions are necessary. Producers and veterinarians recognize the economic significance of NIP sows because these animals contribute to increased NPSDs, with unnecessary extra feed and housing costs for 115 or more days.

Numerous causes and conditions contribute to "false pregnancies"; however, pseudopregnancy is difficult to understand, diagnose, or prevent. In brief, pseudopregnancy can occur when all of the embryos are resorbed after the maternal recognition of pregnancy (days 10 to 14 after mating) and before fetal calcification (days 30 to 40 of gestation). Although the embryos are lost, the sow's ovaries continue to function as if the animal were pregnant. Consequently, the sow remains anestrous for a prolonged period. In the extreme version of pseudopregnancy, the NIP sows may exhibit variable degrees of udder development yet subsequently fail to give birth to any piglets, alive or dead. Unfortunately, producers often mistakenly diagnose these animals as pregnant when pregnancy status is assessed with ultrasound instruments or visual inspection.

It is important to consider the potential infectious agents or causes of embryonic loss between days 10 and 35 of pregnancy. Epidemics of porcine parvovirus, porcine pseudorabies virus, and potentially PRRSV infection can cause abrupt reductions in farrowing rates and increased frequency of irregular return to estrus (outside the typical 21-day interval) and pseudopregnancy. Moreover, infectious agent outbreaks typically induce any of

several other reproductive problems (e.g., abortions, stillbirths, mummification) that accompany the pseudopregnancy.

Increased conception failures and increased rate of embryonic death often result from elevated ambient temperatures. The highest embryonic mortality rate appears to be associated with heat stress in early pregnancy (before 16 days).[17,18] Because the maternal recognition of pregnancy has commenced around the time of embryonic loss, the sow will either return to estrus, experience a reduction in litter size, or demonstrate pseudopregnancy. In addition to heat stress, other stressors, such as regrouping sows at 2 weeks after breeding, may interfere with successful embryo implantation and maintenance of early pregnancy. Although scientific, controlled studies have not been conducted to demonstrate the association between regrouping or animal movement and embryo loss, many producers redesign animal flow to minimize animal handling for the first 21 days after mating.

Other potential causes of prolonged anestrus in sows include cystic ovaries, zearalenone (an estrogenic mycotoxin) toxicity, and endometritis or metritis. These conditions often interfere with the normal estrous cycle of a sow, resulting in erroneous classification of sows as pregnant. Our previous research indicated that less than 20% of NIP sows are affected with any of these conditions, and producers should not overrate their importance.[19]

Regardless of cause, NIP or pseudopregnant sows do not carry pigs to term. Pregnancy detection at approximately 35 days after mating should identify most of these open animals. Reevaluation at days 50 to 60 may reveal true pregnancy status; however, owing to the inherent error associated with pregnancy detection techniques, repeated attempts to identify these animals often are unsuccessful. The specific reasons for the repeated false positive results of pregnancy tests remain debatable.

Through several research projects designed to provide the best pregnancy diagnosis technique, it quickly was determined that no single method is 100% foolproof.[20] A combination of estrus detection at 21 days after mating and two or three ultrasound scans provides acceptable results. With improved technology and instrumentation, real-time ultrasound scanning has proved to be an effective method of visualizing the pregnant uterus. Although the initial cost of real-time ultrasound instruments appears excessive, these instruments commonly are used on large sow farms and integrated production systems.

The incidence of NIP sows varies from farm to farm and from season to season. Because these sows represent an economic loss on certain farms, practitioners need to determine the incidence and probable causes of the condition. Obviously, accurate records are essential to characterize the significance of NIP sows in commercial farms. Improved pregnancy detection methods will assist producer efforts to accurately identify open sows long before the expected farrowing dates.

SUMMARY AND CONCLUSIONS

Sound management of the breeding herd in all phases of production provides the most economical approach to control estrus, ovulation, and conception and farrowing rates. Boar exposure and a thorough understanding of the behavioral and physical changes associated with estrus are effective management tools. It is amazing to note that despite decades of research designed to improve sow reproductive performance, the optimal method to manipulate the porcine estrous cycle and ovulation is to provide male-to-female interactions. Furthermore, today's advanced knowledge of the reproductive physiology of the female pig has not proved to be as effective as competent breeding herd management by experienced personnel.

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CHAPTER 103

Parturition and Dystocia in Swine

ROSS P. COWART

NORMAL PARTURITION

Normal parturition (or farrowing) in swine follows a gestational period of 111 to 117 days. Mean gestation length varies slightly among farms. Variation may be associated with breed or genetic differences and differences in way the beginning of pregnancy is recorded (e.g., first day of estrus, first mating, last mating). The mean gestation length of sows on most farms is 114 to 115 days with a standard deviation of 1.5 days.

Gestation and parturition in the sow are controlled by a complex interaction of physiologic and endocrine events. Although much is known about the hormonal changes that occur during gestation and parturition in the sow, the cause and effect relationship of these changes is either unknown or presumptive in most cases.

Maintenance of pregnancy in the sow appears to be dependent on secretion of progesterone (P_4) by corpora lutea on the ovary. Estrogen levels are also increased during pregnancy (especially the last trimester) and may act to support the function of the corpus luteum (CL). Maternal plasma P_4 levels are elevated throughout pregnancy and decline slowly during the last 10 to 14 days of pregnancy. The last 24 to 36 hours before parturition is marked by a more rapid decline in P_4 levels. The decline in P_4 concentrations coincides with the release of endogenous prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus. Either endogenous or exogenous $PGF_{2\alpha}$ will result in the regression of a mature (greater than 12 days of age) CL. This appears to be an important endocrine signal for the initiation of parturition.[1,2]

Coincident with $PGF_{2\alpha}$ release from the uterus is a rise in the protein hormone relaxin. This hormone is synthesized and stored within the CL of pregnancy. Relaxin levels rise from day 110 of gestation and peak 15 hours before the birth of the first piglet. Relaxin acts to soften the cervix and to inhibit myometrial contractions. Relaxin levels decline during the final moments before parturition. This decline apparently frees the myometrium to begin the contractions of labor. More $PGF_{2\alpha}$ is released from the uterus during labor and apparently stimulates the release of oxytocin from the posterior pituitary of the sow. High levels of estrogen during the last month of gestation act to sensitize the myometrium to the contractile effects of oxytocin.[1]

Physical changes in the sow may signal impending parturition. Astute observers may notice abdominal distention as early as 80 to 90 days of gestation. Evidence of mammary development may be noted soon afterward. During the last week of gestation, the vulva may become swollen and reddened. The mammary glands become increasingly distended and may leak small amounts of colostrum during the day or two preceding farrowing. Large amounts of colostrum may be expressed from the udder within 6 to 12 hours of farrowing.[3]

During the 12 to 24 hours preceding farrowing, the sow becomes restless and may build a nest if bedding material is available. Alternatively, she may paw or root at the floor and bite at the bars of the farrowing crate. Other common behaviors during the last few hours before farrowing include frequent defecation and urination and an increase in respiratory rate. During the last 15 to 60 minutes before the first pig is born, physical activity will decrease and the sow will lie down in lateral recumbency. Abdominal straining may be evident and small amounts of fluid and meconium may flow from the

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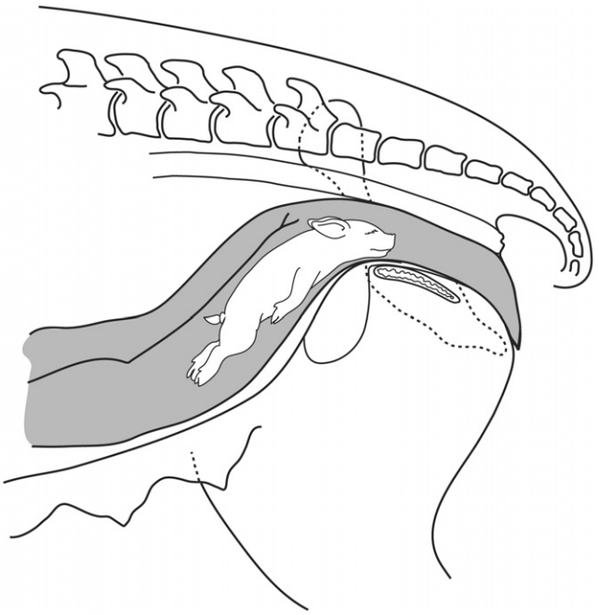

Fig. 103-1 Normal cranial presentation of piglet.

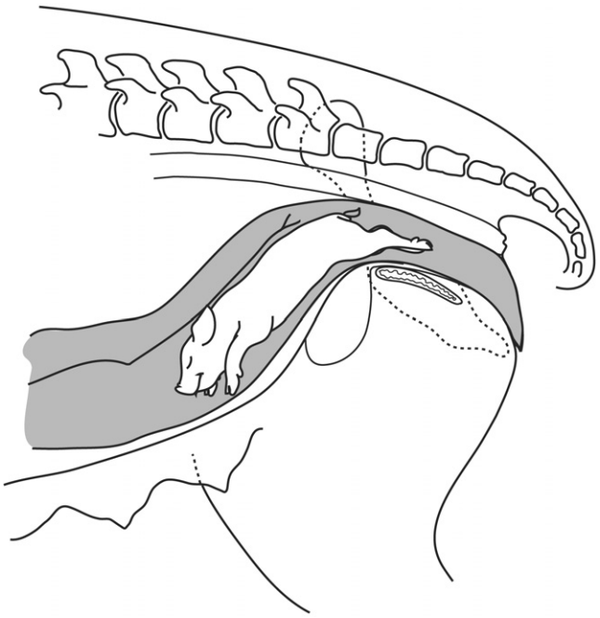

Fig. 103-2 Normal caudal presentation of piglet.

vulva. The first piglet usually is delivered within 15 to 20 minutes.[3]

The duration of farrowing is variable but usually is complete in 1 to 5 hours. The interval between expulsion of successive piglets varies from seconds to hours but typically is 15 minutes or less. An interval of greater than 15 minutes between piglets is associated with an increased probability that the next piglet will be stillborn. Placentas may be expelled between piglets but more commonly are expelled after the birth of the last piglet in the litter. Piglets may be delivered either in a cranial presentation—head first, with front legs folded back against the body (Fig. 103-1)—or a caudal presentation—rear legs first and extended caudally (Fig. 103-2). Although both presentations are considered normal, slightly more than half of all piglets are delivered in the cranial presentation.[4]

DYSTOCIA

Dystocia is relatively rare in sows. The dystocia rate is estimated to be 1% or less of all farrowings. Despite the low incidence, it is important that veterinarians and farrowing house personnel be familiar with the causes of dystocia and possess skills and strategies for dealing with it when it develops. Dystocia can adversely affect productivity by decreasing the number of liveborn piglets and by increasing sow mortality and culling rates. Dystocia compromises the welfare of affected sows, especially when it is not managed in an timely and expedient manner.

Dystocia usually is manifested by the failure to deliver piglets within an hour or 2 of the onset of labor or an interval longer than 1 hour between delivery of piglets within a litter. Other possible signs of dystocia may include prolonged gestation (beyond 116 days), illness of the sow (anorexia and depression), and discolored or fetid vulvar discharges.[3]

Dystocia is caused by conditions which obstruct or impede the passage of the fetus through the birth canal or by ineffective myometrial contractions (uterine inertia). Obstructive causes of dystocia may relate to the fetus, the maternal pelvis, or a combination of fetal and maternal factors. Primary uterine inertia (failure to begin the second stage of labor) is poorly defined but may be caused by hormonal or nutritional abnormalities of the sow. Uterine inertia more commonly is secondary to obstructive causes of dystocia. Abdominal pressing and myometrial contractions against an obstructed birth canal eventually result in fatigue and exhaustion both generally for the sow and specifically of the myometrium.

A mismatch between the size of the fetus and the maternal pelvis is a common cause of obstructive dystocia. In a normal presentation, the shoulders are the largest part of the fetus. If the fetus is especially large or if the maternal pelvis is especially small, the fetus may become lodged at the shoulders in an otherwise normal presentation. Fetuses tend to be larger when the number of piglets in the litter is few. Fetal anomalies, though rare, may result in abnormally large piglets. Maternal pelvic size is smaller in gilts than in mature sows. Some sows have a narrow, V-shaped pelvis (as felt by rectal or vaginal palpation) or lumps protruding into the birth canal from the pubic symphysis (W-shaped pelvis), both of which reduce the functional diameter of the birth canal. Old injuries, such as pelvic fractures, may have the same effect.

Other conditions unrelated to the bony structure of the maternal pelvis may reduce the functional diameter of the birth canal. Constipation or presence of excessive fecal material in the colon and rectum may result in partial occlusion of the birth canal. A full urinary bladder

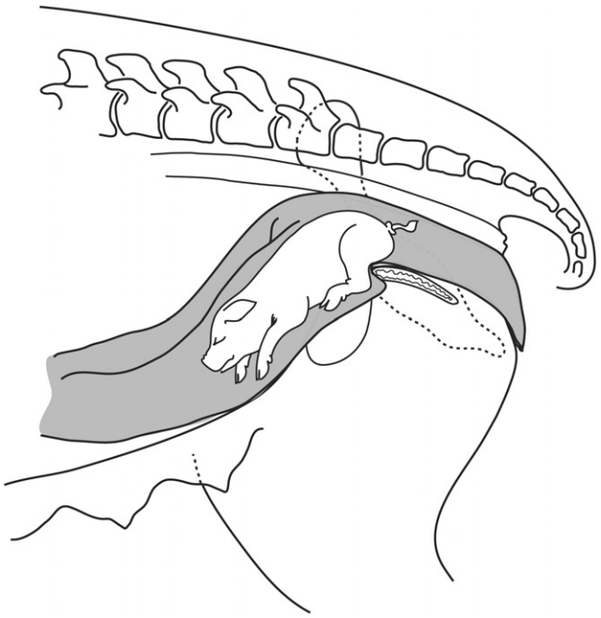

Fig. 103-3 Breech presentation of piglet.

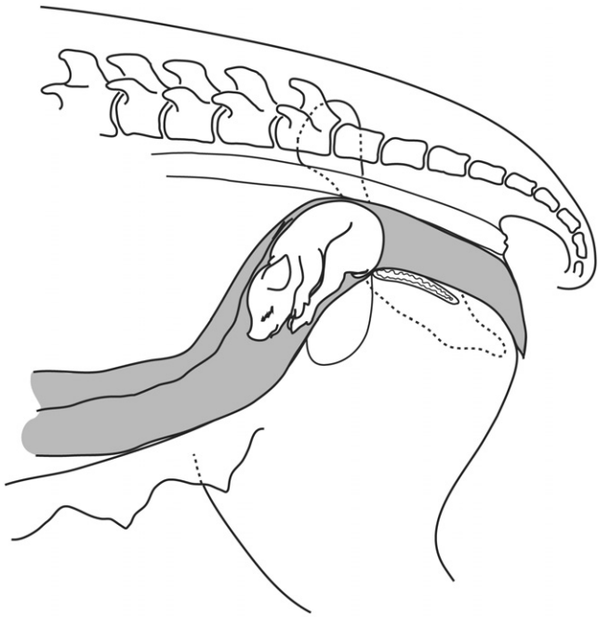

Fig. 103-4 Transverse (sideways) presentation of piglet.

may have a similar effect. Obese sows may have fat deposits in the birth canal that decrease its diameter. Excessive manipulation and examination of the genital tract during dystocia may result in trauma and swelling of the vaginal tissues, particularly if these procedures are performed with inadequate lubrication and gentleness. A persistent remnant of the hymen also may partially occlude the birth canal.

Abnormal posture or presentation of the fetus in the birth canal also may cause obstructive dystocia. In the breech presentation (Fig. 103-3), the fetus is proceeding backward or tail first through the birth canal. This differs from the normal caudal presentation in that the rear legs are flexed forward. In this position, the fetal hocks often catch on the brim of the maternal pelvis, impeding normal delivery. Occasionally, the fetus presents in a transverse or sideways position (Fig. 103-4). In this presentation the body of the fetus is flexed at the backbone or side and this part of the fetus becomes lodged in the birth canal. In the poll presentation (Fig. 103-5), the fetus comes head-first through the birth canal; however, the neck is flexed such that the top of the head (or poll) becomes lodged in the birth canal. In older sows with large litters, the uterus sometimes will be pulled down along the ventral abdominal wall and form an S-curve, with the lower part of the curve going underneath the brim of the pelvis (Fig. 103-6). A fetus may become lodged in this curve underneath the pelvis. On occasion, two fetuses may present in the birth canal at the same time (Fig. 103-7). The maternal pelvis usually is too small to allow the passage of more than one fetus at a time.[3]

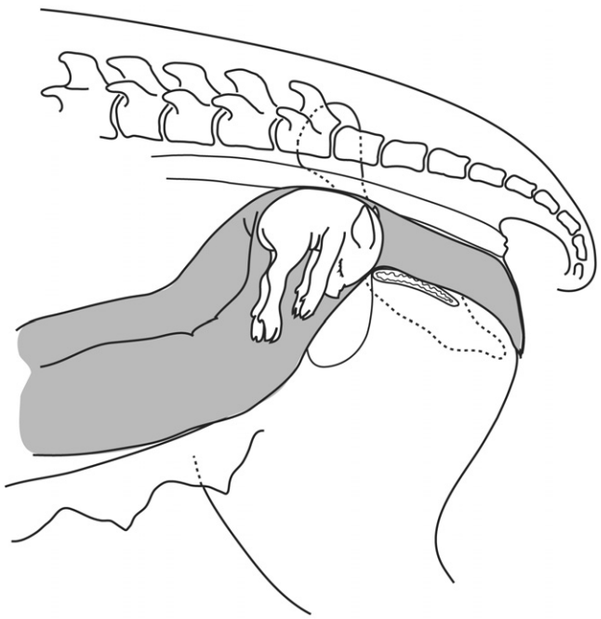

Fig. 103-5 Poll presentation of piglet.

Obstetric Intervention Strategies for Dystocia

Each farrowing enterprise should have a standard protocol in place for obstetric intervention. Although farrowing house personnel are expected to have primary responsibility for attending and assisting sows during parturition, the attending veterinarian should understand and have previously reviewed the protocol for that farm. This is especially important if the protocol includes the use of veterinary prescription drugs, such as oxytocin. Protocols may be individualized for each farrowing unit and should take into account the abilities and training of farrowing house personnel, the welfare of the sow, and the economic realities of intervention strategies.

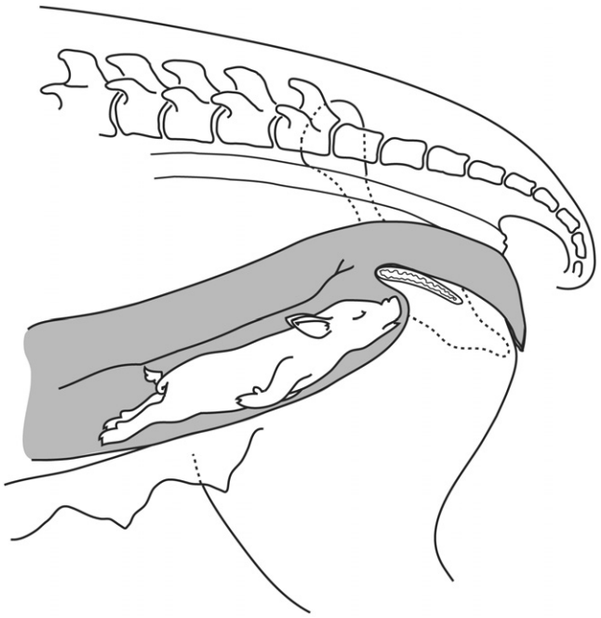

Fig. 103-6 S-curve in uterus of the sow.

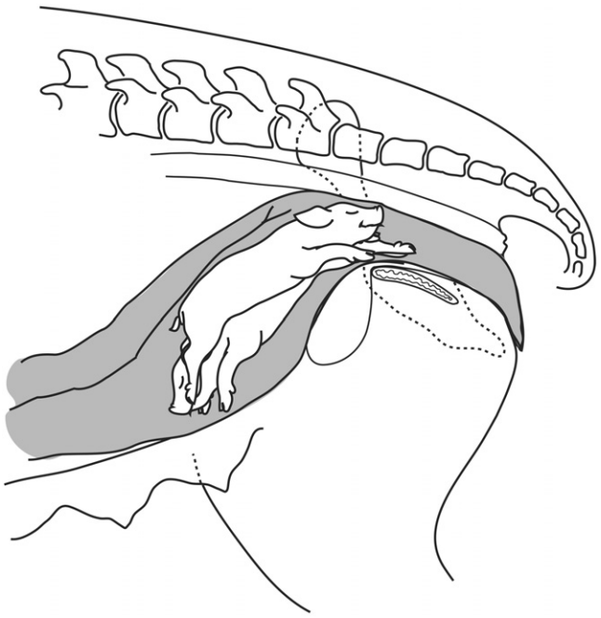

Fig. 103-7 Two fetuses presented at the same time at parturition in a sow.

The farrowing attendant should be observant and knowledgeable about the normal signs of impending parturition. Intervention should be considered if signs of parturition are evident (especially abdominal straining) and no piglet has been delivered for 45 minutes, or if the interval between the birth of piglets exceeds 45 minutes. Similarly, intervention should be considered if the gestation length exceeds 116 days, if the sow is near parturition and appears distressed, depressed, or anorexic, or if abnormal or malodorous vaginal discharges are noted.[3]

The first step of intervention should be a manual examination of the birth canal. The perineal area of the sow and the hands and arms of the attendant should be cleaned with water and antiseptic soap. The attendant should wear a disposable, shoulder-length plastic glove. The attendant's fingernails should be clipped short and the hands and arms should be free of watches or jewelry. A generous amount of obstetric lubricant should be applied to the gloved hand and arm of the attendant and to the vulva of the sow.

The two watchwords regarding obstetric intervention in sows are gentleness and lubrication. Violation of either of these principles can result in significant injury to the genital tract. Such injury may further decrease the probability of liveborn piglets and most certainly results in suffering for the sow. Farrowing attendants with small hands and arms have a distinct advantage when assisting sows with dystocia. Smaller hands can manipulate a fetus with greater dexterity in the limited space of a sow's genital tract and are likely to induce less traumatic injury to the sow.

The attendant should extend the fingers with the tips together so that the hand forms a cone and gently insert the hand into the vagina. The vagina normally courses dorsally from the vulva before passing through the pelvic inlet. Slow, deliberate, and gentle movements will minimize distress in the sow. An attendant with small or average-sized hands should be able reach through the birth canal and into the caudal parts of the uterus in a normal-sized sow. Factors associated with narrowing of the birth canal, such as constipation or a full urinary bladder, should be noted. Manual evacuation of the rectum or catheterization of the urinary bladder may be attempted if appropriate. Removal of the sow from the crate and mild exercise may encourage voluntary voiding of feces and urine.

If a fetus is encountered on manual examination of the birth canal, its position and posture should be determined. If the fetus is in a normal cranial or caudal presentation, the head or rear legs can be grasped by the attendant and gentle traction applied. This often will result in a successful delivery of the fetus. Deposition of obstetric lubricant in the birth canal with a flexible tube or bulb syringe often will make delivery easier and minimize trauma to the sow's genital tract. Abnormal presentations sometimes can be corrected by pushing the fetus farther into the uterus to create more room and then manipulating the fetus into a normal presentation. For example, the fetus in the breech position can be pushed forward and the rear legs grasped and extended into the birth canal, thereby converting the presentation into a normal caudal presentation. The fetus in the poll presentation can be pushed forward and the head and neck extended to result in a normal cranial presentation. Correction of fetal lie may be more difficult with the transverse presentation, but the principles are the same. The fetus is pushed forward and attempts are made to extend either the head or the rear legs into the birth canal.

Most dystocias can be relieved by manipulation and manual traction. In some instances, however, the fetus can be manipulated into a normal presentation, but room in the birth canal is still insufficient to allow passage of the fetus along with the attendant's hand grasping the fetus. In these cases, obstetric snares or forceps may be used. These instruments allow traction on the fetus without adding significantly to the mass or diameter of the fetus. The attendant should be trained in the use of these instruments, or call someone who is trained in their use, to avoid unnecessary injury to the sow or piglets. These instruments allow a great amount of extractive force to be applied and can severely damage the fetus and the sow's genital tract. Copious amounts of lubricant deposited directly into the birth canal and a gentle touch are essential in using these instruments.

An obstetric snare is a smooth wire cable with a loop on the end (Fig. 103-8). The loop can be worked around the head, snout, or hips of the fetus and tightened for a firm grip. A popular pig obstetric forceps consists of two hinged stainless steel rods formed into a handle at one end and a half-moon or semicircular clamp at the other end (Fig. 103-9). The clamping end can be opened on the hinges and worked around the head or hips of the fetus. Then, as the handles swing together, the half-moon clamp closes to firmly grip the fetus. Other clamps and forceps are available but tend to induce more trauma to the fetus than that associated with use of the snare or half-moon forceps.

If a fetus cannot be manipulated into a normal delivery position or extracted through the vagina, then the only remaining options are cesarean section and euthanasia of the sow. The decision between these options must take into account the welfare of the sow and the economic impact of each option. Delaying this decision probably will result in the death and putrefaction of the fetuses remaining in the uterus, with resulting exhaustion, toxemia, circulatory collapse, and death of the sow occurring during the next few hours to days. Therefore, delaying the decision is clearly not in the best interest of the welfare of the sow. Although a decision for euthanasia is onerous and the task unpleasant, it is much superior to no decision at all.

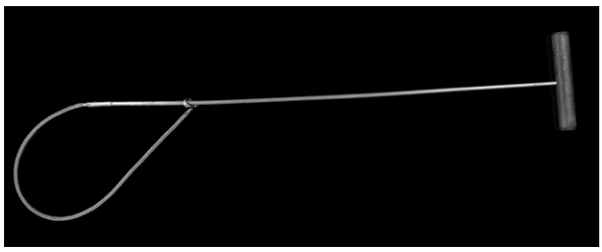

Fig. 103-8 Obstetric snare.

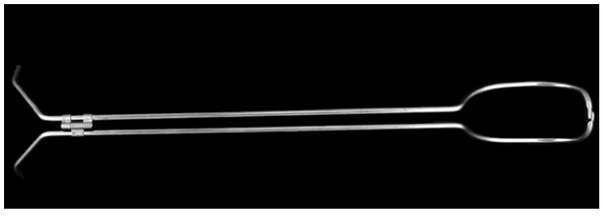

Fig. 103-9 Half-moon obstetric clamp

If a fetus is not encountered on manual examination, then the attendant must determine if (1) the sow is not actually ready to farrow, or (2) the sow is farrowing but the fetuses are farther down in the uterine horns and out of the reach of the attendant, or (3) the sow has completed the farrowing process. If the sow is not ready to farrow, the attendant will encounter a closed cervix approximately 12 to 18 inches inside the vagina. If the attendant's hand passes freely into the uterus, then either the sow has completed the farrowing process or more fetuses are out of the reach of the attendant.

Unfortunately, no practical and conclusive way exists to confirm that the farrowing process is complete. The horns of the uterus are too long to permit complete manual examination. Although radiography or abdominal ultrasound examination may reveal the presence or absence of more fetuses, these techniques are rarely practical on the farm. In most cases, the completion of farrowing is subjectively and fairly accurately assessed by considering the number of piglets born, the delivery of placentas, and the cessation of labor. A majority of sows will farrow at least 5 piglets and expel the placentas soon after the last piglet is born. Exceptions to both of these "rules" will be encountered, however.

Oxytocin is an endogenous pituitary hormone that stimulates myometrial contractions and milk letdown. Exogenous injections of oxytocin will have the same effect. The practitioner should never give injections of oxytocin without first performing a manual examination of the birth canal. Giving oxytocin to a sow with a closed cervix or an obstructive dystocia is ineffective and possibly dangerous. If the cervix is open, however, and no fetus is obstructing the birth canal, then an injection of oxytocin is indicated to stimulate uterine contractions and facilitate the movement of fetuses toward the birth canal. Dosages from 5 to 50 IU have been recommended, by either the intramuscular, subcutaneous, or intravenous route. The low to middle range of those dosages is effective and less likely to induce uterine tetany that is refractory to subsequent injections.[5]

Cesarean Section

Cesarean section offers a reasonable prognosis for survival of the sow and a potential for liveborn piglets if performed within a few hours of the onset of labor. Prognosis declines dramatically as time elapses and the sow shows signs of toxemia and shock. A decision for cesarean section should take into account the condition and prognosis of the sow, the availability of a capable veterinary surgeon within a reasonable time frame, the availability of a reasonably sanitary place to perform the surgery, the costs of surgery, the potential future or salvage value of the sow, and the value and probability of liveborn piglets.

Cesarean section must be performed with use of a local or a general anesthetic technique, or both in certain

instances. The administration of lidocaine into the lumbosacral epidural space will provide analgesia to structures caudal to the umbilicus. The recommended dosage is 1 ml of 2% lidocaine for each 9 kg of body weight, up to a maximum of 20 ml.[6] Alternatively, lidocaine may be infiltrated into the subcutaneous tissues along the incision site. Injectable sedatives or anesthetics usually are indicated to reduce apprehension and mobility of the sow. A number of injectable anesthetic protocols are described.[6] My preference is a combination of xylazine, ketamine, tiletamine, and zolazepam at a concentration of 50 mg/ml for each drug given, either by intramuscular or intravenous injection, to effect the desired level of sedation or anesthesia. This mixture is prepared by adding 2.5 ml each of 100 mg/ml ketamine and 100 mg/ml xylazine to a vial of dry, unreconstituted tiletamine-zolazepam preparation (Telazol*). A dosage rate of 1 ml/13.6 kg (30 lb) has been suggested[7]; however, lower doses may be effective if local anesthetics also are used. None of the suggested injectable anesthetics is currently approved by the United States Food and Drug Administration for administration to swine. Precautions appropriate for extralabel drug use, including extended preslaughter withdrawal times, should be observed. Intravenous administration of isotonic fluids such as saline or lactated Ringer's solution through a catheter in an ear vein may be beneficial, especially in those sows experiencing circulatory compromise due to toxemia.

The sow should be restrained in either right or left lateral recumbency. The uppermost rear leg should be pulled up (adducted) or caudally to expose the lower flank. The surgical site should be prepared in accordance with standard aseptic technique. A 20- to 25-cm incision is made slightly dorsal and parallel to the udder and underneath the fold of the flank. The incision is extended through the skin, subcutaneous tissues, abdominal musculature, and peritoneum. A gravid uterine horn is located and exteriorized and laid on a sterile drape or plastic sheet. A longitudinal incision is made in the uterine horn midway between the ovary and the bifurcation of the uterus. Ideally, all fetuses in one uterine horn can be delivered through a single incision; however, multiple incisions occasionally are necessary if the fetuses cannot be manipulated toward the incision site. The uterus should be carefully examined to ensure that no fetuses have been overlooked. Fetuses located near the bifurcation of the uterus are especially easy to overlook. When all of the fetuses have been removed from the horn, the serosal surface is rinsed with sterile isotonic saline and closed with a continuous inverting suture pattern using absorbable suture material. The procedure is repeated with the opposite uterine horn. The peritoneum and abdominal musculature are closed with absorbable suture material using a pattern of the surgeon's choice. The skin may be closed with a nonabsorbable suture material using an external pattern or with absorbable suture material using a subcuticular pattern. The latter choice obviates the need for suture removal 10 to 14 days later. Oxytocin may be administered postoperatively to enhance uterine contraction and evacuation. Systemic antibiotics may be administered if contamination from the environment or from putrefying fetuses is believed to be a problem.[6]

If a decision for cesarean section cannot justified on the basis of welfare or economics, then euthanasia should be promptly performed. Recommended procedures for on-farm euthanasia of swine have been published in a brochure available from the National Pork Board and the American Association of Swine Veterinarians.[8]

PHARMACOLOGIC INDUCTION OF FARROWING

Most sows normally will farrow within 2 days of a mean gestation period of 115 days (measured from the first day of estrus). A narrow time window of farrowing is helpful in scheduling the use of facilities and in ensuring that attendants are available to assist if necessary when the sow is farrowing. Although synchrony of breeding is the most important method in limiting the time window for farrowing, pharmacologic induction of farrowing can add further precision to the time of farrowing.

The administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or an analogue will safely and effectively induce farrowing in sows after day 110 of gestation. Most sows will begin farrowing between 20 and 36 hours after $PGF_{2\alpha}$ administration. Therefore, if the objective is to have a majority of sows farrowing during a typical 8 AM to 5 PM workday, when attendants will be available, administration of $PGF_{2\alpha}$ should occur the previous morning. Few adverse effects are observed if sows are at least 111 days into gestation. If sows are at less than 110 days of gestation when treated, piglet viability will be compromised. Therefore, accurate breeding records are essential when pharmacologic induction of farrowing will be used.[5]

The only prostaglandin compound currently approved in the United States for the induction of parturition in swine is dinoprost tromethamine (Lutalyse*). A dose of 10 mg given by intramuscular injection to sows within 3 days of expected farrowing is indicated for induction of farrowing. This drug is available only by the prescription of a licensed veterinarian. Care should be exercised to avoid human exposure to this drug because injection or cutaneous exposure may result in bronchospasm or abortion. Although experimental data for prostaglandin analogues such as cloprostenol suggest safety and efficacy, these compounds are not approved for use in the United States.

Synchrony of farrowings is increased when oxytocin is given to sows 20 to 24 hours after administration of $PGF_{2\alpha}$. Injection of 5 to 30 international units (IU) of oxytocin 20 hours after $PGF_{2\alpha}$ injection will result in farrowing in a higher percentage of sows in the next 6 hours than if $PGF_{2\alpha}$ is given alone. Therefore, a reasonable protocol for induction of farrowing would include the injection of $PGF_{2\alpha}$ at noon the day before the targeted farrowing date and injection of oxytocin at 8:00 AM on the targeted farrowing date. Although oxytocin adminis-

*Fort Dodge Laboratories, Inc., Fort Dodge, IA.

*Pharmacia & Upjohn, Kalamazoo, MI.

tration at the higher end of the dosage range (20 to 30 IU) is more effective for synchronizing farrowings, these doses may induce uterine tetany and fatigue. The result would be a higher percentage of sows requiring obstetric intervention and a higher risk of stillbirths in the piglets and of genital injury and infection in the sow. Therefore, if oxytocin is included in the protocol for induction of farrowing, doses at the lower end of the range (5 to 10 IU) are recommended.[5]

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CHAPTER 104

Postpartum Care of the Sow and Neonates

THOMAS J. FANGMAN and SANDRA F. AMASS

Swine production managers today find both personal and financial satisfaction in maintaining a comfortable and sanitary environment for the sows and pigs under their care. This requires the manager to be particularly attentive to the comfort of individual sows, which includes monitoring feed and water intake and environmental conditions. Profit and productivity depend on ensuring that piglets receive adequate colostrum and milk so that a large number of healthy pigs can be weaned from each sow that farrows.[1]

GESTATING FEMALE CARE

Ensuring that gestating gilts and sows are comfortable and adequately fed facilitates the farrowing event. The gestating sow should be fed to maintain good body condition without overconditioning (obesity). The gestation ration formulated for sows must be balanced to provide the daily nutrient requirement of sows as they progress through the stages of pregnancy. Gilt gestation rations should be optimized to provide adequate protein, energy, calcium, and phosphorus for growth of the gilt and her unborn litter.

Nutrient restriction is used to optimize weight gain in sows. Today's National Research Council (NRC) requirements for gestational sows utilize many factors to consider weight gain.[2] This weight gain is influenced by the gestating sow's requirement for energy, maintenance, protein accretion, fat accretion, products of conception, and thermoregulation.

Amino acid requirements of gestating sows are influenced by their maintenance requirements, protein deposition in proteinaceous tissues, and protein deposition in the products of conception. Folic acid and biotin can be used to supplement gestation rations for increasing the number of liveborn pigs.

Water should not be restricted for gestating females. Low water intakes can predispose female pigs to cystitis.

Use of gestation crates enhances body condition by allowing individual females to be fed according to their

tration at the higher end of the dosage range (20 to 30 IU) is more effective for synchronizing farrowings, these doses may induce uterine tetany and fatigue. The result would be a higher percentage of sows requiring obstetric intervention and a higher risk of stillbirths in the piglets and of genital injury and infection in the sow. Therefore, if oxytocin is included in the protocol for induction of farrowing, doses at the lower end of the range (5 to 10 IU) are recommended.[5]

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Water should not be restricted for gestating females. Low water intakes can predispose female pigs to cystitis.

Use of gestation crates enhances body condition by allowing individual females to be fed according to their

needs. Pen gestation can result in variable body condition when smaller females cannot compete for feed. These pigs lose weight, whereas dominant females tend toward obesity.

In some production systems, protection of neonatal pigs from infectious diseases (e.g., infection due to *Clostridium* spp. or *Escherichia coli*) is required. To this end, generally the pregnant females are vaccinated once at 4 to 6 weeks and again at 2 weeks before farrowing for neonatal diseases endemic to the herd. Protection from pathogens can be partially controlled by ensuring that piglets receive high levels of protective immunoglobulins from the colostrum of their dam.

Controlling external and internal parasites commonly involves treatment of the sow approximately 2 weeks before farrowing to prevent pigs from being infected by their dams and to avoid contamination of the farrowing crate with expelled parasite eggs or larvae. Gestating females need to be identified, and individual sow records should be located in close proximity to the sow. Good records will ensure that sows are moved to the farrowing room 1 to 3 days before parturition. Extremely high piglet mortality is observed when litters are born in the gestation barn. The typical gestation length ranges from 113 to 117 days in most herds.

Pig flow can become uneven when management abandons an all-in–all-out strategy, resulting in insufficient time to sanitize the farrowing room. Farrowing in the gestation barn may occur with increased frequency, especially if the number of sows farrowing is large.

Gilts may require 3 to 4 days to adapt to the farrowing crate. This additional adaptation time may prevent agitation in gilts and reduce the incidence of piglet crushing and savaging.[3]

All sows entering the farrowing house should be examined, with particular emphasis placed on the underline of the sow. Damaged mammary glands or inverted nipples should be noted and recorded, because this information can be utilized in making cross-fostering and culling decisions.

It is tentatively assumed in the absence of convincing data that sows should be washed with soap or mild detergent and water to remove pathogen-containing manure before entry into the farrowing house. This assumption, however, requires further testing.

The farrowing facility generally is the most costly facility of the production system and requires the greatest labor specialization. Therefore, many production systems will have less than 48 hours to clean rooms in between groups, even though a longer time between groups may reduce contamination by bacterial pathogens in the barn. Sanitation of the farrowing facilities is crucial to preventing neonatal pig scours. To minimize neonatal disease, farrowing facilities should be managed on an all-in–all-out basis to allow thorough cleaning, disinfecting, and drying between groups.

Using flooring that provides a high proportion of open space to solid space that is easy to keep clean and dry can minimize pathogen exposure.[4] This flooring generally is made of cast iron or steel rods that may be coated. Plastic flooring also is used. Utilization of cast iron or steel flooring under the sow and plastic-coated wire or plastic flooring in the creep area can improve piglet comfort and decrease preweaning mortality by keeping the sow cool and piglets warm. Piglets will avoid being laid on by the sow by staying away from the "cool" sow flooring.

The number of sows that can be farrowed at any given time often limits the number of pigs produced. The number of pigs weaned per farrowing crate is a good measure of how a farrowing facility is being utilized. Farms weaning pigs before 21 days of age should have a goal of 140 pigs or more weaned per farrowing crate per year, or 9.5 weaned pigs per sow.[5]

CARE OF THE SOW AT FARROWING

Paying particular attention to the comfort of the sow at farrowing is paramount to the profitability of a production facility. Good animal husbandry skills are rewarded at this phase of production by increased sow survivability and employee satisfaction. Farrowing is a time of high risk for sows. It has been observed that 42% of sow deaths occur during the peripartum period, and an additional 16.5% of sow death loss occurs during lactation.[6] During the peripartum period, sows are prone to specific disease conditions, such as mastitis and metritis, that may lead to lactation insufficiency. Heat stress is common in sows during the hot months of summer, resulting in a seasonal increase in death loss. Uterine prolapse in the sow is rare but often is fatal; it accounts for less than 7% of all sow death losses.[6]

As the sow begins parturition, she will become partially or completely anorectic for several hours before and after parturition. Respiratory rate will increase 30 to 80 minutes before parturition to 95 to 105 breaths per minute (normal 13 to 18 per minute) and rectal temperature has been observed to increase from 38.7° to 40.0°C 24 hours before farrowing. Pigs are usually born at 15-minute intervals. Intervention is recommended if the farrowing interval is longer than 1 hour between pigs. The most common cause of dystocia is uterine inertia; however, sows should be manually examined using a hygienic technique before any treatments are administered. Oxytocin can be administered at a dose of 5 to 10IU every 2 to 4 hours to control uterine inertia after it is established that a piglet is not lodged in the birth canal. Larger doses of oxytocin can inhibit the desired effect.

Good animal husbandry practices include the following:

- Minimizing loud or sudden noises or disruptive activities around sows, especially piglet processing and castration in adjacent farrowing crates
- Ensuring the comfort of the sow, which may require drip cooling during the summer months
- Encouraging the sow to stand and drink water when it is apparent that farrowing is completed and the placentas have been passed
- Providing a farrowing crate design, particularly for the lower portion of the crate, that does not

interfere with the sow's ability to rise and lie down or obstruct the upper row of teats

POST-FARROWING CARE OF THE SOW

When the sow stops straining and begins to demonstrate an interest in her litter, the farrowing attendant can assume that farrowing is complete. Complete expulsion of the fetal membranes and placentas is the final phase of parturition, however. The time required for expulsion of the fetal membranes may range from 20 minutes to 12 hours after the last pig is born. Retained placenta occurs rarely in sows. Failure to find the placentas in the farrowing crate 4 to 12 hours post partum suggests the presence of another pig in the birth canal, and a vaginal examination is indicated. Sows that continue to strain, have a malodorous and discolored vulvar discharge, or show signs of depression or weakness also should be vaginally examined for retained pigs.

Many sows are anorectic during parturition and may refuse to eat for the next 48 hours. Feed should be withheld from sows (or only a very small amount provided) the day of farrowing. Then feed can be increased to 4 pounds daily, plus 1 pound per pig per day for the first week, with an average intake of 10 to 12 pounds of feed per day. Water intake is essential for optimizing feed intake and milk production during lactation. Lactating sows will drink 4 to 5 gallons of water per day, and the recommended flow rate for nipple waterers is 2 quarts per minute.

The sow is continually available for suckling by the newborn pigs for the first few hours after parturition. This constant mammary stimulation results in a high level of circulating oxytocin and facilitates the piglet's ability to readily obtain colostrum. The sow generally is exhausted from parturition and demonstrates little interest in the piglets. During this time, however, some sows are observed to savage their newborn pigs. This condition tends to occur more often in primiparous sows, and the aggressive behavior is often directed toward the first-born piglet. Separation of the piglets from the sow until farrowing is completed usually is all that is required to calm a sow that is savaging her piglets. On some occasions a sow may require sedation before accepting her piglets or fostered piglets. The sow's udder should be inspected for color, consistency, heat, and lesions likely to be associated with pain at this time to determine if the sow is suffering from mastitis or any other puerperal disease condition.

Approximately 24 hours after birth the sow will begin to actively encourage the pigs to nurse by grunting and positioning her mammary glands so that the nipples are available for suckling. Cyclic nursing begins at this time, and milk letdown occurs approximately every hour for a period of a few minutes.

POST-FARROWING CARE OF THE NEONATAL PIG

Newborn pigs require immediate energy intake and must be provided a microenvironment that is draft free and dry with a temperature of at least 30°C.[7] Heat loss can be reduced if piglets are dried at the time of birth or shortly after by temporary placement of an additional heat lamp at the rear of the crate. Piglets acquire immunoglobulins from colostrum. It is imperative that newborn pigs suckle within the first few hours after birth. Colostral immunoglobulin G (IgG) levels drop by 50% within 6 hours of the first nursing; late-born piglets may receive significantly lower levels of passive immunity than littermates born earlier in the farrowing order.[8] When the piglets are 24 hours old, the small intestine loses its ability to transport immunoglobulins (macromolecules) to the lymphatic system, and "gut closure" occurs. It is a good practice to collect excess colostrum from newly farrowed sows and store it in the freezer for the purpose of supplementing weak or orphaned piglets.

As piglets are born, an effort should be made to dry each animal and dip the umbilical cord into a mild disinfectant solution. Clipping of needle teeth usually is performed to reduce damage to the sow's underline and to minimize wounds sustained by piglets when fighting to establish dominance. Some producers in Europe and the United States do not clip needle teeth and observe no adverse effects. The decision to clip needle teeth will vary according to farm-specific conditions. Piglets should be allowed to suckle colostrum before their teeth are clipped. Further piglet processing usually occurs at 3 to 5 days of age. Processing tools should be sharp and should be disinfected in between litters. Sick litters should be processed last.

Pigs have a limited iron supply at birth, and sow's milk provides very little iron. Without iron supplementation, piglets will develop a microcytic anemia within 2 weeks of birth. To prevent microcytic anemia in piglets, it generally is recommended to administer an intramuscular injection of 200 mg of iron dextran in the neck of each piglet within the first 5 days after birth.

Taildocking often is performed at the same time the iron is administered so that any pigs that have not received an iron injection can be easily identified. Tails can be trimmed with side-cutting pliers to a length of about 2 cm from the body. Taildocking is performed to reduce the incidence of tail biting in the grow-finish stage of production. Ear notching or tattooing also can be performed before the pig reaches 5 days of age. Male pigs should be castrated between 5 and 14 days of age.[9]

Cross-fostering is the practice of moving pigs between litters to achieve uniform weight and to ensure that adequate functional teats are available to the number of pigs suckling. This practice is particularly important for sows with pendulous udders, which may not be able to expose the bottom row of teats to their piglets. Pigs should be moved from one litter to the next within the first 24 hours after birth so that the fostered pig can receive colostrum from its new dam. Care should be taken to avoid placing all small pigs on primiparous sows because the small pigs may not provide the young sow with aggressive-enough stimulation to ensure oxytocin release. Pigs can be bottle-fed, or mechanical feeding systems can be used. Feeding pigs milk replacers requires a great deal of additional labor to maintain a high level of sanitation of the equipment. Cost and labor considerations should be thoroughly evaluated before any decision regarding acquisition of a mechanical feeding system.

Providing additional attention to individual pigs can be rewarding. Warming individual pigs that become chilled or have limited mobility and providing nourishment by means of a stomach tube can give them a head start before they are placed with their littermates.

Splay-legged piglets can be assisted by providing support tape between their two rear legs. This tape should allow the animal to walk with short steps and can be removed in 2 days, allowing the pig to stand without assistance.

REDUCING PREWEANING MORTALITY

Significant improvements in management and housing have reduced baby pig mortality on many farms. From 15% to 25% of all piglets die during farrowing or within the first few weeks of life, however.[10] The death loss from birth to weaning is one of the major contributing factors limiting herd productivity and profitability.[10] Causes of neonatal mortality are tightly interlinked. Major death losses due to crushing and starvation probably reflect a variety of etiologic factors. Piglet losses tend to be the result of noninfectious causes and are strongly associated with management practices. Epidemics of certain neonatal diseases can occur and may result in extremely high mortality rates for limited periods of time. The most common causes of neonatal death include stillbirth, trauma, low viability, chilling, starvation, and diarrhea.

Most piglets that are born dead die of anoxia caused by premature rupture of the umbilical cord. Some decrease in blood flow to the fetus is common during normal uterine contractions at farrowing. More serious reductions can occur through damage or occlusion of the umbilicus, or with placental detachment. The resulting hypoxia can result in stillbirths and reduced postnatal viability. Hypoxia increases the amount of time between birth and first suckling and is associated with hypothermia, reduced postnatal growth, and higher neonatal mortality rates. Stillbirths are more common with older sows and among large litters and the last pigs born in a litter.[11] The number of stillborn pigs at each farrowing may vary, ranging from 5% to 10%, but can be higher on occasion. Manual intervention during prolonged farrowings is the most effective means to reduce the incidence of stillbirths. Such intervention should be conducted in a sanitary fashion and with a gentle touch, however, to avoid trauma to the sow. Females can be induced to farrow so that the number of observed farrowings is increased, and assistance provided when necessary.

Low birth weight is the greatest contributing factor to low viability of piglets. Approximately 30% to 40% of pigs that weigh less than 900 g at birth die during the first 3 weeks of life. In some production systems, a management decision is made to remove these very small piglets from the system. If it is determined to be feasible to raise these small pigs, then an intensive postnatal management plan will need to be instituted. This plan should include drying the piglets quickly after birth and moving them to a heat source to conserve their scarce energy reserves. Small, weak pigs may need assistance to nurse or may require colostrum and milk given by a stomach tube. A synchronized farrowing schedule combined with a proper cross-fostering program within the first 24 hours after birth will ensure litters of even weights and appropriate numbers for the nursing capacity of the sows. Placing all small pigs on a gilt or first-parity sow should be avoided because these piglets may not be strong enough to aggressively stimulate the dam's mammary gland, resulting in decreased milk letdown.

Chilling results when the environmental temperature drops below the pig's lower critical temperature and the animal must increase its metabolic rate. This critical temperature for baby pigs has been determined to be 34° C.[7] The pig's viability, activity, and vigor are greatly reduced when its core body temperature drops 2° C below this critical temperature. This chilling effect is observed more frequently in smaller pigs because they have a greater surface area–to–body weight ratio, resulting in greater heat loss. Air drafts of any kind must be minimized, because exposure to drafts will further reduce the effective temperature of the pig's environment. Cold pigs will not suckle and will subsequently die from hypoglycemia.

Neonatal pigs instinctively seek warmth at birth and often lie next to the sow's udder. A heat lamp placed behind the sow at farrowing is a good management practice to reduce chilling of wet newborn pigs. Placement of a heat lamp on either side of the farrowing crate encourages pigs to move away from the udder and reduces the number of pigs that may get laid on by the sow.[12]

Although slightly more males than females are born, females have a greater survival advantage than males.[13]

Sows lying on or crushing pigs is the most common cause of death in pigs in the first 3 days of life.[11] The incidence of crushing can be greatly reduced with the use of a well-designed farrowing crate. The crate design should encourage the sow to adopt a position of sternal recumbency before proceeding to lateral recumbency. Another important feature of crate design is nonslip flooring that will allow good mobility of the piglets and ensure that the sow can get up and down easily. Provide the sow with a comfortable resting environment and reduce sow irritation, anxiety and periods of restlessness by feeding at regular intervals (up to four times per day) and by avoiding loud or unfamiliar noises.

Infectious diseases of the alimentary tract are the most common type of disease in the suckling pig. Losses due to diarrhea can account for 5% to 15% of preweaning deaths.[14]

Enteropathogenic *E. coli* is the most important infectious cause of disease in neonatal pigs. These bacteria possess pili or fimbriae that enable the organism to adhere to the small intestines. Once *E. coli* has attached to the mucosa of the small intestine, it will induce hypersecretion of fluids and electrolytes by means of enterotoxins. Enteropathogenic *E. coli* infection can cause diarrhea in pigs by 12 hours of age. Control measures are directed at reducing piglet exposure to the organism through good hygiene and sanitation practices and by maximizing the passive immunity protection that piglets receive from sow's colostrum and milk. Effective commercial bacterins are available. Sows can be vaccinated before farrowing so that protective antibodies will be passed on to the piglets. Any disease in the sow that disrupts milk supply, such as mastitis (see Chapter 105), will

place the suckling litter at greater risk of infection by enterotoxigenic *E. coli*.

Coccidiosis in pigs is caused by *Isospora suis* and commonly is seen in pigs 7 to 14 days of age but can occur in pigs as young as 5 days of age. Mortality rate from coccidiosis usually is low, but diarrhea caused by this organism results in decreased growth rate and can affect a large number of the pigs exposed. Diagnosis can be made by identifying large numbers of *I. suis* merozoites in smears from intestinal mucosa. Control of disease due to this organism is best accomplished by strict sanitation measures including disinfection with 50% bleach and by using perforated flooring materials for raised farrowing crates. Currently, no coccidiostats are approved for use in swine in the United States.

Clostridium perfringens type C is regarded as an important swine pathogen in some production areas of the Midwestern United States. This organism classically will cause peracute enteritis in pigs that are 12 to 36 hours old; acute enteritis when pigs are approximately 3 days old; subacute enteritis when the pigs are 5 to 7 days old; and chronic enteritis when pigs are 1 to 3 weeks old. Lesions range in nature from severe hemorrhagic scours in peracute cases to thickened bowel with a diphtheritic membrane in chronic enteritis. Antibiotic therapy, antitoxin, and vaccination can be used in control programs combined with strict sanitation measures.

Viral diarrhea of piglets often is associated with a very sudden and dramatic increase in preweaning mortality rates and also can be a cause of endemic disease losses. Common viral etiologic agents include transmissible gastroenteritis (TGE) virus, porcine epidemic diarrhea virus, and rotavirus. Disease due to these viruses should be suspected whenever the small intestine of a suckling pig is thin and pale. An epidemic of TGE infection is characterized by vomiting and diarrhea affecting all pigs from 2 days of age to adult, with the greatest morbidity and mortality observed in the youngest animals exposed. Natural infection provides an extended high level of immunity in adult swine. Immune sows can protect their pigs with antibodies in colostrum and milk. In herds in which transmissible gastroenteritis is enzootic, vaccination of the sow before farrowing sometimes can help protect suckling pigs.

Systemic infections represent only a small proportion of death loss in neonatal pigs. Streptococci and *E. coli* are the main bacterial causes of septicemic disease during the neonatal period. Polyarthritis commonly leads to failure to thrive and may occasionally be a cause of death. Organisms commonly isolated from the joints of these pigs include groups C and L streptococci, *Actinomyces pyogenes, Staphylococcus* spp., and *E. coli*. Ensuring good colostrum intake by all pigs, ensuring ready access to milk throughout the nursing period, and hygienic processing procedures can prevent polyarthritis and systemic infections. Providing a clean and sanitary environment also will reduce the likelihood of systemic disease. Treatment with antibiotics can be successful if initiated very early in the course of the disease, but prevention through improved management is preferred.

Several important nonenteric viral diseases can affect suckling pigs. Porcine reproductive and respiratory syndrome virus (PRRSV) can cause death of affected piglets and is associated primarily with premature farrowings, weak-born pigs, and death loss due to respiratory disease (principally interstitial pneumonia) and secondary bacterial infections. Pseudorabies also can be an important cause of high piglet mortality during an acute outbreak but can be controlled by vaccination or natural immunity.

In addition to infectious agents, sudden increases in preweaning mortality rate can be caused by environment, husbandry, and nutritional factors. The most important factors to consider in dealing with an increase in preweaning mortality rate are the skill and attitude of the farrowing room stockperson. The best design features of a farrowing facility are those that make it easy for dedicated stock people to perform their duties to the best of their abilities.[15]

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CHAPTER 105

Diseases of the Puerperal Period

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Diseases of the puerperal period often have been referred to collectively as a syndrome consisting of **mastitis, metritis,** and **agalactia,** also known as the **MMA complex.** These three clinical entities can occur at the same time, but in many production systems it is common to see either mastitis or metritis followed by agalactia. Infections of the urinary tract also may lead to the production of endotoxins and serve as a source of bacteria that may lead to metritis and subsequent agalactia. Agalactia also has been be referred to as *lactation failure, postpartum dysgalactia,* and *preparturient hypogalactia syndrome.* The variety of names for this condition suggests a wide range of potential etiologic disorders, with variable levels of severity that may be unique to each production system.

A thoroughly cleaned and disinfected farrowing area that provides comfort to the sow or gilt is critical for preventing infections during the puerperal period, when these animals are tired and stressed from parturition. This period is characterized by an increased susceptibility to the development of cystitis, mastitis, metritis, and agalactia. Once the dam has become infected, the availability of milk to the litter will be reduced, with a consequent decrease in piglet survival.

This chapter reviews the potential causes of mastitis, metritis, cystitis, and lactational insufficiency syndrome, as well as the interactions among etiologic factors. Treatment and prevention protocols are described when appropriate.

COLIFORM MASTITIS

Mastitis describes any pain, swelling, or inflammation of the mammary gland. Coliform mastitis is an infectious condition caused by a variety of gram-negative organisms.[1] This condition is observed commonly in swine production systems. The increase in confinement housing during breeding, gestation, and farrowing has increased the comfort of the animal but also has increased the need for management practices emphasizing sanitation to reduce environmental bacterial exposure. Although *Escherichia coli* has been the most commonly encountered bacterial isolate in cases of coliform mastitis, other gram-negative bacteria such as *Klebsiella pneumoniae, Citrobacter freundii,* and *Enterobacter aerogenes* also have been suggested as possible pathogens.[1] Noncoliform organisms are occasionally isolated (*Staphylococcus epidermidis* or *Streptococcus* spp.). The small number of reported clinical cases involving gram-positive organisms, however, indicates that gram-negative bacteria are much more significant etiologic agents.[1] Systemic signs observed in mastitic sows are primarily the result of absorbed endotoxins and the formation of inflammatory endogenous mediators in the mammary gland.[2] Endotoxin has been observed to suppress the release of prolactin, resulting in a subsequent decrease in milk production.[3]

Epidemiology and Clinical Signs

Coliforms are part of the normal microflora of swine. These organisms have been isolated from udder, teat, urinary bladder, and uterine discharge.[4] Coliforms commonly are isolated from swine feces, suggesting that the source of infection may be from the sow or a contaminated, nonsanitized environment. Contamination of teats takes place within 2 hours after farrowing. Bacteria rapidly colonize the ductular or alveolar lumina of the mammary gland.

Clinical manifestations of coliform mastitis in sows include fever (40° to 42° C), anorexia, cachexia, malaise, and agalactia. The udder is erythematous and edematous. In severe cases of coliform mastitis, clinical signs may include induration and discoloration of the mammary glands, which may pit and blanch when digital pressure is applied. Sows may display behaviors indicative of high levels of pain when the gland is palpated. In some cases, constipation and mammary edema may be present. A purulent exudate often can be expressed from the nipples of affected glands, and single glands or gland complexes can be affected. Septicemia or toxemia may result in elevated respiratory and heart rates. Sows with coliform mastitis often lie in ventral recumbency and demonstrate a reluctance to allow piglets to nurse. This condition rapidly leads to lactation insufficiency, which is discussed in greater detail later in the chapter.

Diagnosis

Gross lesions of coliform mastitis are confined to the mammary gland and regional lymph nodes.[1] Postmortem findings include regional lymphadenopathy, and longitudinal sections of udders reveal scattered areas of firmness, which are gray to red in color.[1] Histologic lesions vary in severity within the same mammary gland, ranging from a small number of neutrophils in the alveolar lumina to severe purulent infiltration with necrosis. Inguinal and iliac lymph nodes may be affected with acute purulent lymphadenitis.[1] Laboratory tests used

for detection of mastitis in cows are not commonly employed in sows because of the naturally elevated cell count in sow's milk.[1] Culture of material from affected glands is difficult, and specimens are easily contaminated by bacteria on the skin of the sow or the practitioner. Contamination can be reduced by wearing sterile gloves or by surgical preparation of the udder with tamed iodine solutions. Samples should be inoculated onto sheep blood agar and MacConkey's agar and incubated for 24 hours at 37° C. Biochemical tests can be carried out to determine the identity of the organism isolated.

Treatment and Prevention

In acute cases of coliform mastitis, aggressive therapy is indicated. Antibiotic selection should be based on culture and sensitivity results. Response to antibiotics, however, is complicated by the heterogeneous pattern of antimicrobial susceptibility of individual isolates, not only within the herd, but also in the individual sow.[1] The pharmacokinetics of antimicrobials for mastitis therapy in swine has received only limited attention. Other compounds that may be administered in conjunction with antibiotics include prednisolone (50 to 100 mg), oxytocin (30 IU), and B vitamins to stimulate the appetite.[1] It also may be helpful to administer a prostaglandin synthetase inhibitor such as flunixin meglumine. Care of the piglets may require fostering or provision of a milk substitute (discussed later under Lactation Insufficiency Syndrome).

The best preventive measures include proper hygiene, especially removal of fecal material during and after parturition.[5] The farrowing room and sow area should be cleaned with hot water (temperature greater than 95° C), thoroughly disinfected, and allowed to dry before the sows are moved in. It is a good management practice to wash sows with a mild soap and water, with particular attention given to the mammary area, before entry into the farrowing room. If manual intervention is required during parturition, attendants should thoroughly wash their hands and arms, wear well-lubricated arm-length sleeves, and wash the perineal region of the sow before manually entry into the birth canal. All sows requiring manual assistance during parturition should be identified and treated with an effective antibiotic. Rectal temperatures should be taken two or three times a day after treatment. Those sows that demonstrate a rectal temperature higher than 40° C should be treated for 3 to 5 days.

Currently, no vaccines for prevention of mastitis are commercially available, and positive reports from the use of experimental products have been scarce. Accordingly, the most cost-effective measures for control appear to be the management factors described here.

METRITIS AND URINARY TRACT INFECTIONS

Metritis is characterized by inflammation of all layers of the uterus. Clinical signs of metritis are commonly seen 24 to 48 hours after parturition. The urinary tract may act as a source of endotoxins and bacteria that can infect the genital tract, leading to metritis.

Epidemiology and Clinical Signs

The same factors that predispose sows to the development of urinary tract infection also may increase their susceptibility to metritis. The numerous microbes that normally inhabit the porcine reproductive tract also can be found in swine manure and have the potential to be pathogens at parturition.[5] During gestation, the uterus is under the influence of high levels of progesterone secretions. This progesterone influence results in a closed cervix, generalized immune suppression, and an increased bacterial population.[6]

Contamination of the environment along with excessive microbial proliferation can result in infection of the uterus. Metritis also can be the result of overzealous infusion practices and excessive, unsanitary manual intervention during parturition.

Cases of metritis are characterized by a necrotic, malodorous discharge that frequently will contain remnants of fetal membranes. The sow often is anorectic, febrile, and septic. Septicemia in these cases often will result in death. Milk flow is minimal, and piglet mortality rate is increased.[5]

Diagnosis

Metritis can be readily diagnosed by the appearance of a vaginal discharge and the malodorous nature of this discharge. At postmortem examination, the uterine lumen may be filled with necrotic, yellow-brownish, purulent material.[5] Histologic evaluation generally will demonstrate edema and purulent exudate in several layers of the myometrium.[6] Colonies of bacteria may be evident microscopically as abscesses within the myometrium. Chronic cases are characterized by a diffuse infiltration of lymphocytes and denuded endometrial epithelium.[6] Culture of an affected uterus commonly results in isolation of *E. coli, Staphylococcus aureus,* or *Actinomyces pyogenes*.[5]

Material for uterine culture in a live animal is readily obtained if the cervix is patent. Culture specimens can be obtained using uterine swabs inserted through a vaginal speculum.[5] Reproductive slaughter checks also may serve as an opportunity to obtain tissue and bacterial isolates during an increased incidence of metritis. Vaginal cultures provide little information owing to the high level of contamination of this area with numerous species of nonpathogenic bacteria.[5]

Treatment and Prevention

Antibiotics for therapy of the infection should be selected on the basis of in vitro sensitivity and administered systemically. Intrauterine infusions of sodium penicillin or oxytetracycline produce satisfactory concentrations of these antibiotics in the uterine lumen. Intrauterine therapy is not widely accepted. Problems of uneven drug distribution exist. Some evidence also suggests that intrauterine infusion of antibiotics with a narrow spectrum of antibacterial activity has resulted in the elimination of an individual organism (such as *E. coli*), only to result in the abnormal proliferation of other bacteria,

which may prolong the required time for regeneration of the endometrial lining after parturition.[7]

The steps involved in the prevention of metritis are similar to those described for preventing coliform mastitis. Suspected cases of urinary tract infection or endometritis should be treated with an antibiotic selected on the basis of culture and sensitivity testing results, a history of effectiveness on the farm, or practice experience. The most important factor is proper hygiene before, during, and after parturition at a time when the cervix is open to pathogen invasion. It is critical to recognize that the fecal bacterial flora are the same etiologic agents of metritis.

LACTATION INSUFFICIENCY SYNDROME

Lactation insufficiency (LI) is a condition in which milk production and flow are inadequate to maintain the viability of the litter. This section focuses on the noninfectious causes of this syndrome and the effects of this syndrome on the pig. Rarely is there a total failure of lactation. Suboptimal milk production, however, is relatively common. Sows with generalized suboptimal milk production and individual gland mastitis will often raise normal litters, with one or two pigs that rapidly fall behind their littermates in development. Entire litters may linger without starving and dying but do not gain weight and grow as do other litters.

Epidemiology and Clinical Signs

Regardless of etiology, the primary clinical signs of lactation insufficiency are observed in the baby pigs, rather than the sow. Piglets become restless and hungry and rapidly lose body condition. Piglets will be vocal and nuzzle the udder aggressively. Eventually, they will become gaunt and dehydrated and die almost exclusively during the first 3 days of life.

The risk of diarrhea may be increased by 90% in litters for which milk production is suboptimal. In these cases, the causative organisms may be identified, but the underlying etiology is frequently not discovered.

Noninfectious causes of LI have traditionally been classified as heritable, nutritional, or endocrine imbalances and as those related to management or environment.[8] A genetic predisposition to LI is likely to exist but difficult to prove. Within breeds, certain lines or families of sows tend to demonstrate a higher incidence of LI. Those sows that are affected with LI once are more likely to have the same condition in subsequent lactations. Endocrine imbalances have been documented as contributing to LI. Such imbalances are more likely to occur as a result of endotoxemia, however, than as a primary cause of LI.

Ergot intoxication caused by *Claviceps purpurea* is rare but can result in LI in a group or herd of sows fed grains contaminated with this organism.

Other noninfectious causes of LI generally affecting individual animals include teat malformations (inverted nipples), psychogenic agalactia (pig savaging), and mammary gland edema.[8] The primary noninfectious categories of causes of LI are management/environment and nutrition/feeding.

More cases of LI are observed in sows that farrow and lactate on solid concrete floors. Sows that have difficulty farrowing, have large litters, or deliver mummified pigs are more likely to suffer from LI. Sows that are moved from a gestation pasture to a confinement farrowing facility immediately before farrowing, without an adjustment period, also are predisposed to the development of LI. Heat stress and other environmental stressors that decrease appetite also precipitate LI. A seasonal peak in the occurrence of LI also has been observed in the third quarter of the year in the United States.

Diagnosis

Clinical manifestation of disease in affected sows varies widely depending on the specific etiologic factors. Those cases that are caused by noninfectious agents are associated with few pathologic changes in the sow. In pigs the pathologic lesions will be characteristic of starvation. Lack of body fat, lack of digesta in the stomach and intestinal tract, and diarrhea often are features in pigs from affected litters.

Treatment and Prevention

Treatment of infectious cases of LI is directed toward the elimination of the cause, as well as alleviation of the clinical signs. Oxytocin (5 to 10 U) should be administered to stimulate milk letdown. This therapy can be repeated every hour for up to 6 hours, or at 2- to 4-hour intervals during the first day of treatment. Sows affected with LI thought to be of noninfectious origin should be treated with oxytocin initially. If mammary edema is present, a diuretic such as furosemide or a glucocorticoid may be indicated. Glucocorticoids should be avoided when coliform mastitis is suspected. Induction of parturition with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been found to reduce the incidence of LI. It appears that $PGF_{2\alpha}$ induces an immediate increase in prolactin concentrations and may prevent premature mammary gland engorgement.

During the periods of agalactia, the piglets need particular attention. Energy-rich nutritional supplements and electrolytes should be administered, and if diarrhea is present, antibiotics should be administered. Piglets can be dosed with a stomach tube. Caution should be used to avoid causing aspiration pneumonia, however. Small amounts (2 to 5 ml) of supplement should be given every 1 to 2 hours until normal milk flow resumes.

FEEDING/NUTRITION

What happens in one phase of the sow's cycle influences her productivity in subsequent reproduction phases. For instance, a sow with low voluntary feed intake (less than 10 pounds/day) and nursing a large litter (more than 10 pigs) during lactation will lose body weight. This lactating sow will more than likely to wean a litter with a high weaning weight but more unlikely to rebreed promptly after weaning. Therefore, the sow's nutritional state in one phase of her cycle (lactation) influences the next phase of her reproductive cycle (rebreeding).

Gilt Development

A successful reproductive program begins early in the gilt's life to ensure complete preparation for a long productive life in the breeding herd. Feeding gilts for efficient, rapid lean gain allows accurate assessment of the female's growth potential and enables sufficient development of body tissues that will be needed later in her reproductive life. Beginning at 45 kg of body weight, dietary calcium and phosphorus should be increased by 0.1% above typical grow-finish diets to enhance skeletal development of gilts. The recommended dietary nutrient requirements for replacement gilts are a minimum of 0.8% calcium (18g/day) and 0.7% phosphorus (15 g/day).

Controlled growth is the target once females enter the gilt pool at about 150 days of age. Restricted feeding (2.3 to 2.8 kg) of a moderate-energy (3300 kcal ME/kg), high-protein (16%) diet will slow body growth and, more important, will reduce deposition of fat tissue while allowing continuous growth of lean body mass. In recent research, such a diet significantly increased the proportion of gilts that had a first litter and stayed in the breeding herd long enough to farrow a fourth litter.[9] Diets for females in the gilt pool contain the same vitamin and mineral fortification as in the diets for older sows in the breeding herd. Because feed intake is restricted in animals entering the gilt pool, gilts must receive a flushing level of feed intake 10 to 14 days before breeding to maximize ovulation rates. Feed intake needs to be reduced to pre-flushing levels immediately after breeding to avoid high embryo mortality rates common with high post-breeding feed intake.[9]

Feeding Strategies during Gestation

During gestation, the feeding and management practices focus on preparing the sow for parturition, fetal growth, and lactation performance. In the very early stages of gestation immediately after conception, the first objective is to provide conditions that will increase the likelihood of maximal survival of embryos and ensure a large litter size at the subsequent farrowing. Growth of the developing fetuses and increasing nutrient stores in the sow's body throughout growth toward mature body size, or replenishment of stores lost during the previous lactation, are the main objectives during mid-lactation (days 30 to 75). In late gestation, fetal growth continues at a very rapid rate, and mammary development occurs in preparation for the upcoming lactation. Growth of the products of conception is fairly resistant to nutritional manipulations at feed intakes typical of production settings during late gestation. Under conditions of adequate energy intakes (6 to 10 Mcal ME daily), changes in weight of fetuses at birth are relatively small and have little influence on body composition. Some research indicates that increasing energy feed intake of pregnant primiparous gilts increases total weight of fetuses at birth, but not in multiparous sows. The energy requirement for the products of conception has been set at 35.8 kcal ME daily for each fetus.[10] The desired outcome of a successful gestation feeding program is a large, vigorous litter of pigs and a healthy sow equipped with the adequate mammary development and body stores of nutrients to produce large quantities of the milk for the litter.

It has been well documented that excess energy intake during gestation decreases feed intake during lactation and causes a reduction in milk production and increased tissue catabolism. Several researchers have demonstrated the inverse relationship between feed intake during gestation and feed intake during lactation. As gestation feed intake increases, subsequent lactation feed intake decreases, and loss of body weight and backfat increases. The reduction in feed intake is thought to be due to reduced glucose tolerance and insulin resistance caused by excess feed intake during gestation. The decreased insulin resistance causes mobilization of stored nutrients and leads to high concentrations of circulating energy substrates in the plasma. Essentially, affected sows do not receive the metabolic signals that demand more nutrient intake. The reduction in feed intake is especially pronounced during the first week of lactation, a critical time for establishing milk production levels and subsequent reproductive function. Feed intake during gestation should be moderate (1.8 to 2.25 kg/day) to prevent depressed feed intake and excess tissue catabolism during lactation. Minimum daily allowances for amino acids, vitamins, and minerals must be maintained when energy intake is limited during gestation.

Calcium and phosphorus are very important to gestating sows for development of fetuses in utero and integrity of the sow's skeleton. A reduction in bone mineralization may compromise longevity of sows in the breeding herd owing to a higher incidence of crippling injuries and increased proportion of nonbreeders. Proper mineral nutrition during gestation can replenish bone mineral reserves that were depleted during lactation to satisfy the high mineral demands of milk production.[11] Daily intakes of 14 to 18 g of calcium and 12 to 16 g of total phosphorus during gestation seem to satisfy the reproducing sow's needs.

Mammary Development before Lactation

Body composition in late gestation may affect mammary development and ultimately milk production in the subsequent lactation. Very little mammary development occurs during gestation until day 75. After day 75, an exponential growth occurs in the concentration[12] and total quantity[13] of DNA and RNA in the mammary gland. DNA content is a measure of the total number of cells, whereas RNA content provides an indication of the protein synthetic capacity of a tissue. When dietary energy intake of gilts was increased from 5.75 to 10.5 Mcal ME daily after day 75 of gestation, a reduction in the weight and total DNA and RNA of parenchymal tissue in the mammary gland was observed, indicating that excess energy has the potential to reduce the capacity for milk synthesis.[13] Researchers reported that the percentage of protein, fat, ash, and DNA in each suckled mammary gland was affected only by total energy intake.[14] In contrast with the effects of elevated energy intake, altering protein intake of gilts during late gestation had no influence on mammary development.[13] Increasing lysine

intake from severely deficient (4 g/day) to liberal (16 g/day) concentrations[10] had no effect on number of milk-secreting cells in the mammary gland[15] but did increase milk production in the subsequent lactation.[16] As litter size increases, the total mammary gland weight and protein, fat, ash, and DNA contents increased, resulting in an additional required 0.96 g of lysine/day to account for the mammary gland growth for each pig added to a litter ranging in size from 6 to 12 pigs.[17] Therefore, providing adequate amounts of nutrients (energy and protein) to sows during lactation relative to litter size is important for achieving maximal growth of mammary gland and milk production. Ultimately, mammary gland growth is affected by nutrient intake during late gestation and early lactation.

Energy and Protein Needs during Lactation

Lactation is a particularly important stage of the reproductive cycle and ultimately places a tremendous nutrient demand on the sow. Lactating sows will produce milk quantities equal to 10% or more of their body weight daily. Therefore, the lactating sow produces as much dry matter output in 2 days as a pregnant sow produces in 114 days. Dietary nutrients need to meet the high milk production demand through either dietary intake or tissue catabolism. Proper nutrition is critical in order to avoid limiting milk production and to prevent excessive tissue catabolism, which can lead to poor reproductive performance after weaning. Long-term sow productivity depends on maintenance of body condition and ensuring optimal nutritional status relative to metabolic demand.

Energy intake during gestation is not a major cause of lactation failures, unless a severe energy deficiency occurs over an extended period of time. Inadequate energy intake during lactation, however, can reduce milk production and increase sow body weight loss. Increasing the energy density of the lactation diet (with supplemental fat) or increasing total feed intake can increase energy intake during lactation. The supplemental fat is used preferentially by the mammary gland to increase milk fat and increase litter weaning weight. Unfortunately, the added fat will not substantially reduce sow weight or backfat loss.

Lysine is the first limiting essential amino acid in most diets for lactating sows. The estimated total lysine requirements for the lactating sow range from 32 to 58 g/day. The amino acid requirements during lactation can be divided into the requirements for maintenance and the requirements for milk production. The maintenance requirement of the sow during lactation is relatively small. For example, an average lactating sow will require 2 to 3 g/day and 50 to 60 g/day of lysine for maintenance and milk production, respectively. The recommendations for amino acids are closely correlated with the level of sow productivity achieved, and lactating sows require 26 g/day of lysine for each kilogram of daily litter weight gain. These high amino acid requirements can be met only through nutrient intake and catabolism of body protein. Loss of body protein during lactation, however, may influence subsequent reproduction of sows.

Amino acid intake during lactation has a major impact on sow milk production and litter-weaning weight of primiparous sows.[18] It was not until recently that research indicated that increasing amino acid intake from 32.3 g/day to 73.3 g/day decreases voluntary feed intake during lactation and decreases subsequent litter size.[19] The lysine intake required to minimize protein loss (59 g/day) during the first lactation decreases subsequent total number of piglets born compared with the lysine intake required to maximize milk production (32 g/day). Thus, the goal during lactation is to feed adequate protein concentrations to maximize litter growth rate (44, 55, and 56 g/day lysine intake for sows of parity 1, 2, and 3, respectively) and only slightly reduce muscle catabolism. Overall, maximizing feed intake during lactation is critical to enhancing lactation performance and subsequent reproduction cycles.

Conclusions

An effective nutrition program for the sow herd must be based on sound nutritional principles. There are no "magic bullets" that allow producers to achieve high biologic performance while ignoring basic nutritional concepts. Every effort must be made to ensure that nutrient requirements are estimated accurately, feeding strategies are implemented properly, quality control programs are practiced, and results are monitored regularly. A sound feeding program begins early in the sow's life. Continual attention to specifics of the feeding program is necessary because nutrition in one phase of the reproductive cycle sets the stage for sow performance in the next phase of production. Many factors influence productivity of the swine breeding herd. Genetics, nutrition, health status, housing, and management all blend together to establish the level of productivity in a breeding herd. Just as factors interact to influence breeding herd productivity, phases of the sow's reproductive cycle interact. Any failures in the nutritional program of the breeding herd, however, could lead to increased incidence of diseases during the puerperal period.

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CHAPTER 106

Bacterial, Rickettsial, Protozoal, and Fungal Causes of Infertility and Abortion in Swine

MONTSERRAT TORREMORRELL

Losses due to reproductive failure are high in today's swine industry. Many factors are involved in causing reproductive failure, including infectious agents, such as bacteria, viruses, protozoa, and fungi, and noninfectious factors, such as nutrition, genetics, environment, management practices, and husbandry procedures. This chapter reviews infectious causes of reproductive failure—primarily diseases caused by bacteria, protozoa, rickettsiae, and fungi. Such diseases have become less important in today's high-technology swine industry with the improvement of the sanitation procedures and the widespread use of artificial insemination. Some of the diseases discussed in this chapter are still considered important at the individual animal level and in specific herds, but for the most part such diseases are considered sporadic in the U.S. swine industry.

Bacteremias, septicemias, toxemias, and viremias exert their effects directly when microorganisms invade the bloodstream and pose a threat to the life of the conceptus or the dam, or indirectly through mechanisms to induce fever. The degree to which these diseases influence the conceptus depends on the stage of pregnancy and the virulence of the organisms. In order to understand the clinical signs associated with reproductive failure in swine, it is important to review some of the terminology used and understand the stages of embryo and fetus development. **Gestation**, which is the period between conception and parturition, lasts about 114 days in swine.

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CHAPTER 106

Bacterial, Rickettsial, Protozoal, and Fungal Causes of Infertility and Abortion in Swine

MONTSERRAT TORREMORRELL

Losses due to reproductive failure are high in today's swine industry. Many factors are involved in causing reproductive failure, including infectious agents, such as bacteria, viruses, protozoa, and fungi, and noninfectious factors, such as nutrition, genetics, environment, management practices, and husbandry procedures. This chapter reviews infectious causes of reproductive failure—primarily diseases caused by bacteria, protozoa, rickettsiae, and fungi. Such diseases have become less important in today's high-technology swine industry with the improvement of the sanitation procedures and the widespread use of artificial insemination. Some of the diseases discussed in this chapter are still considered important at the individual animal level and in specific herds, but for the most part such diseases are considered sporadic in the U.S. swine industry.

Bacteremias, septicemias, toxemias, and viremias exert their effects directly when microorganisms invade the bloodstream and pose a threat to the life of the conceptus or the dam, or indirectly through mechanisms to induce fever. The degree to which these diseases influence the conceptus depends on the stage of pregnancy and the virulence of the organisms. In order to understand the clinical signs associated with reproductive failure in swine, it is important to review some of the terminology used and understand the stages of embryo and fetus development. **Gestation**, which is the period between conception and parturition, lasts about 114 days in swine.

In the first 2 weeks of life, the conceptuses lie free in the uterine lumen. Damage to them during this stage is almost always fatal.[1] **Implantation** begins around day 12 of gestation; death of the conceptus before then will result in resorption of the conceptus, and the sow will undergo a regular return to estrus at approximately 21 days. Death of the embryo before calcification, between approximately 14 and 35 days of gestation, will result in abortion or complete resorption of the embryo. A delayed return to estrus may be observed after 24 to 28 days, resulting in an irregular return to estrus.

Fetal death after day 35 typically is followed by mummification of the fetus, rather than pregnancy termination, because the mineral content of the fetus prevents complete absorption. Mummified fetuses are characterized by discoloration and resorption of fluids and soft tissues.[1]

Abortion refers to the delivery of an immature fetus, either live or dead, before the completion of the gestation period as a result of the failure of the mechanisms that control pregnancy. A **stillbirth** is a fetus that has matured fully in utero but is born dead. Stillborn piglets look normal, but their lungs do not float in water (respiration never occurred before death). Stillbirths may be caused by infectious agents; affected fetuses usually die before the end of gestation and around the **prepartal period.** Stillbirths also may be caused by noninfectious agents or conditions and are known as **intrapartum deaths.**[1] Most common noninfectious causes include hypoxia secondary to umbilical rupture or impairment of umbilical blood flow, high atmospheric carbon monoxide concentration, elevated temperatures (higher than 39° C) during farrowing, and prolonged parturition.[1] **Neonatal death** refers to that occurring primarily within the first 7 days of life. Death usually is due to events that take place during late gestation or shortly after parturition. In production records, neonatal death usually is captured within the preweaning mortality parameter.

Although a few bacteria such as *Leptospira* and *Brucella* are recognized to cause major reproductive economic losses, the reality is that in modern swine production systems these diseases are not significant, and they should be considered exceptional. On the other hand, sporadic losses due to infections caused by ubiquitous bacterial agents, often considered secondary to stresses and management practices, can result in significant economic losses because of the chronic nature and persistence of these diseases, which also typically are difficult to control and diagnose.

LEPTOSPIROSIS

Leptospirosis has been reported as a cause of reproductive loss in swine from all parts of the world.[2] Leptospirosis is caused by a variety of motile, aerobic spirochetes from the *Leptospira* genus. The leptospires that affect animal species (the parasitic strains) are grouped into about 23 serogroups containing approximately 212 serovars based on complicated agglutination reactions.[2] Reproductive signs in susceptible herds are characterized by abortion, birth of dead full-term piglets, weak-born piglets, and infertility.

Epidemiology and Clinical Signs

Leptospires persist in the kidneys and genital tracts of carrier swine. Shedding may occur intermittently for prolonged periods, for up to 2 years. Therefore, the primary mode of transmission of leptospirosis to pigs is through contact with voided urine from infected hosts. Contact of infected urine with the oral, nasal or ocular mucosa will result in a brief period of bacteremia of less than 10 days. During this time, bacteria can be isolated from most organs and fluids of the body, including the cerebrospinal fluid. Once the organism is cleared from the bloodstream, it will take up residence in the proximal tubules of the kidney.

Pigs act as maintenance hosts for serovars belonging to the pomona, australis, and tarassovi serogroups, whereas strains belonging to the canicola, icterohaemorrhagiae, and grippotyphosa serogroups are among the more commonly identified incidental infections in swine.[2]

Leptospira pomona has been the most common serotype isolated from pigs worldwide. Many strains of serovar pomona, especially those of type kennewicki, are adapted to pigs and have been found in the United States and Canada.[3] *L. pomona* is reported to be endemic in North and South America, Australia, New Zealand, and parts of Asia and Eastern and Central Europe, but not Western Europe. Many strains within the pomona serovar have rodent hosts. Transplacental infection occurring during the short period of maternal leptospiremia in the second half of gestation may result in abortion. Chronic leptospirosis also will result in stillbirths and weak-born piglets, with a majority of the fetuses in the litter being affected. *L. pomona* does not appear to cause infertility.

Leptospira bratislava serotype has emerged as a major swine-maintained leptospiral infection in the last few years. Serologic data have indicated widespread infection in Europe, United States, Australia, Brazil, and South Africa. The epidemiology is poorly understood, because evidently many strains are maintained in pigs, dogs, horses, and hedgehogs and other wildlife. Although the renal carrier state does become established, urinary excretion is poor compared with pomona excretion. By contrast, venereal infection is thought to play an important role in the spread of the disease because the bacteria are known to persist in the upper genital tracts of boars and the oviduct and uterus of sows.[2] Abortion caused by *L. bratislava* infection is the result of presence of the leptospires the genital tract. *L. bratislava* has been recognized as a cause of infertility.

The serotype icterohaemorrhagiae is carried by the brown rat *(Rattus norvegicus)*. Although serologic evidence of widespread infection has been reported in many countries, very few isolates are available from pigs.[4] Field investigations suggest that infection between swine is inefficient.[2] The duration of urinary excretion is short (less than 35 days) in naturally infected pigs. In acute episodes of infection, *L. icterohaemorrhagiae* will cause jaundice and hemoglobinuria in young piglets younger than 3 months of age. In chronic episodes, weak-born litters have been reported as a feature of *L. icterohaemorrhagiae* infection.[2]

The serotype grippotyphosa is maintained in wildlife, and swine are considered only an alternate host. Infection in pigs is considered incidental. Some serologic evidence of its presence has been reported in Eastern and Central Europe and the United States, but in general, this serotype is not considered a significant pathogen of swine.[2] Of interest, *L. grippotyphosa* was one of the most common serotypes recovered in a study involving Iowa herds.[3]

The serotype canicola has been recovered from swine in several countries. Little is known of its epidemiology in pigs, however. The dog is the recognized maintenance host for this serovar. *L. canicola* is excreted in pig urine for at least 90 days and has the ability to survive in undiluted urine for at least 6 days, suggesting that between-pig transmission could be possible, although no studies are available in this subject. This serotype can cause abortions, stillbirths, and periods of infertility.[2]

Published information is limited regarding the tarassovi, hardjo, and sejroe serotypes. The pig is considered to be the maintenance host for some of the tarassovi strains found in Eastern Europe and Australia. In the United States, *L. tarassovi* has not been recovered from swine, but serologic evidence of infection has been described in pigs of the southeastern states, where it has been isolated from wildlife (raccoons, skunks, and opossums).[2] *L. hardjo* is maintained worldwide in cattle.[2] The organism has been isolated in pigs in the United Kingdom and the United States. Between-pig transmission is considered to be irrelevant because persistence in renal tissue is very limited. Serovar sejroe has been isolated from swine in Europe and is mostly maintained by small rodents.[2]

Diagnosis

Lesions associated with leptospirosis infection are not serotype specific and are not considered pathognomonic.[2] The primary lesion is damage to the membranes of the endothelial cells of small blood vessels. Lesions are very limited in acute leptospirosis. Petechial and ecchymotic hemorrhages can be seen in the lungs of some pigs. Minor lesions can be seen in the renal tubules. Focal liver necrosis, lymphocytic infiltration of the adrenal glands, and meningoencephalitis with perivascular lymphocytic infiltrations also have been described. In chronic leptospirosis, lesions are confined to the kidneys and consist of scattered small gray foci, often surrounded by a ring of hyperemia. These lesions are characterized by focal interstitial nephritis, manifested as interstitial leukocytic infiltrations by lymphocytes, macrophages, and plasma cells. Focal damage also may involve glomeruli and renal tubules. Bowman's capsule may be thickened, containing eosinophilic granular material. Atrophy, hyperplasia, and presence of necrotic debris may be seen in the renal tubules. Chronic lesions may be noticeable as long as 14 months after infection. Leptospires can invade the pig's mammary gland and produce a mild, focal nonsuppurative mastitis. Lesions in aborted fetuses are nonspecific and may include edema of various tissues, fluid in body cavities, petechial hemorrhages in the renal cortex, and small foci of interstitial nephritis. The liver may exhibit focal necrosis manifested as small grayish-white spots. Placentas from aborted fetuses are grossly normal.

Laboratory procedures most commonly used for the diagnosis of leptospirosis include serologic testing, culture, and immunofluorescence. Serologic testing is the most widely used method, and the microscopic agglutination test (MAT) is the standard serologic test. MAT is considered a herd test. Rising antibodies in acute and convalescent animals constitute a sign of recent infection. Titers of 1:1000 or greater are strongly indicative of recent infection. Chronically infected animals may have titers below the accepted minimum significant value of 1:100, for which this test has very low sensitivity. Presence of antibodies in fetal sera or thoracic fluid is diagnostic of leptospiral abortion. Culture of leptospires from affected tissues is the definitive diagnostic tool. The organism is fastidious, however, so culture is time-consuming, with special requirements, and is not performed on a routine basis. Immunofluorescence testing is the method of choice for diagnosis of fetal leptospirosis in fetal body fluids or kidney smears. Darkfield microscopy of fetal fluids or urine also has been used; however, precautions have to be taken so that the organisms are not confused with tissue artifacts.

Treatment and Control

Prevention of transmission from an infected pig or other hosts, or from infected urine, to a pig is the critical factor in management. Control is achieved by a combination of vaccination, antibiotic treatment, and management factors. Commercially available vaccines are used routinely before breeding and contain five or six serotypes. Vaccines are available in most of the countries with intensive pig production. Vaccination will help reduce the prevalence in an infected herd but will not eliminate infection. Very few antibiotics are available to treat leptospirosis at this time. Feed-grade tetracyclines can be used, but high levels are required (800 g/ton). Streptomycin injections at 25 mg/kg of body weight also may be used. Main management factors for the control of leptospirosis are the prevention of direct or indirect contact with wildlife vectors and with other domestic animals such as pigs, cows, and dogs, or with their urine; maintenance of excellent hygiene; and provision of a dry environment for the herd. Effective rodent control programs should be instigated in and around all pig production complexes. The use of artificial insemination and the addition of antibiotics in the semen extender are important procedures in the control of *L. bratislava* infection. Leptospirosis is a zoonotic disease, and precautions need to be taken accordingly.

BRUCELLOSIS

Brucella suis is the ethologic agent of brucellosis in swine. Brucellosis occurs in most countries throughout the world. Prevalence is decreasing, however, because of improved sanitation, institution of husbandry standards, and national eradication programs. In the United States, brucellosis remains endemic in the South. *B. suis* not only

is a problem in swine in which it causes abortion, orchitis and infertility but also is an important zoonotic agent, with implications for public health.

Epidemiology and Clinical Signs

Pigs become infected through direct contact with aborted fetuses, fetal membranes, and body excretions.[5] Pigs of all ages may become infected. Brucellosis is a venereal disease. *Brucella* organisms can survive in organic material in the environment for prolonged periods at freezing or near-freezing temperatures. *B. suis* is readily killed by pasteurization, sunlight exposure, and most commercially available disinfectants. Feral swine constitute the most important reservoir in the United States and rabbits in Europe.[5]

On penetration of the mucosal epithelium, invading organisms are carried to the local lymph nodes, where they invade neutrophils and macrophages and become established intracellular microorganisms protected from the humoral immune response. A phase of bacteremia follows and may last from 1 to 7 weeks.[5]

The classic manifestations of pig brucellosis are abortion, infertility, orchitis, posterior paralysis, and lameness. Gilts or sows with infections acquired through the genital tract at the time of breeding will abort during the first third of gestation and will have irregular returns to estrus. Little or no vaginal discharge is observed. Abortions also may be seen during the middle and last thirds of gestation with infections acquired during pregnancy past 35 days of gestation. Testicular infection in the boars will result in infertility and lack of sex drive. If accessory genital glands are affected, *B. suis* organisms will be shed in large quantities. Clinical brucellosis in suckling and weaning pigs usually manifests with spondylitis associated with posterior paralysis.[5]

Diagnosis

Gross pathology is variable. Areas of abscessation may be seen in affected organs, which may progress to necrosis and desquamation of the mucous membrane. Focal microscopic granulomatous lesions also may be seen in livers in the acute phase of infection. Boars may experience unilateral testicular enlargement; the affected gland is hard to palpation.

Isolation of *B. suis* in the most sensitive and accurate method to diagnose swine brucellosis. Tissues from aborted fetuses contain large quantities of organisms. *B. suis* also can be cultured from semen, testicles, and accessory organs and from lymph nodes, fluid from swollen joints, and, in the early stages, blood.

A variety of serologic tests are available for blood samples. Agglutination and complement fixation tests, enzyme-linked immunosorbent assays (ELISAs), and card and plate tests are among the most commonly used serologic tests. The brucellosis card test, buffered plate antigen test, and the rose bengal test are the most practical methods at present to conduct large-scale surveillance monitoring. Both false positive and false negative results occur, and serologic testing must be used for herd testing, rather than for diagnosis in individual animals.

Treatment and Prevention

Treatment with antibiotics is not very effective and generally should not be attempted. Affected pigs should be destroyed. Safe and reliable vaccines against brucellosis in pigs are not available. There is a movement in a number of countries aimed at gradual elimination of the disease by compulsory slaughter of affected herds coordinated through national eradication campaigns. Overall, the degree of management of swine herds and the fact that breeding stock companies and artificial insemination centers have to be validated brucellosis free have played a major role in reducing the spread of *B. suis*.

ERYSIPELAS

Swine erysipelas is caused by the gram-positive bacterium *Erysipelothrix rhusiopathiae* and is characterized clinically by fever associated with characteristic diamond-shaped skin lesions, arthritis, vegetative endocarditis, and sudden death and by abortion in pregnant sows.[6]

Epidemiology and Clinical Signs

E. rhusiopathiae is regarded as a commensal organism of swine flora and is found in the upper respiratory tract and the intestinal tract of healthy pigs. Greater than 50% of pigs are considered to be carriers. *E. rhusiopathiae* survives in the soil for prolonged periods and may also be found in other mammalian species including sheep, fish, and birds. *E. rhusiopathiae* is considered to be a zoonotic agent.[7]

Infection is through oronasal exposure. Subsequently, invasion of the bloodstream results in septicemia. *E. rhusiopathiae* infection in swine is characterized by clinical illness with depression, high fevers (temperatures greater than 40°C), diamond-shaped skin lesions, lameness, and endocarditis. Abortions also may occur as a result of the high fevers and are seen in the acute form of the disease. If abortion does not take place, one or two piglets may die inside the womb and become mummified. Abortion may happen in any of the stages of gestation. Absorption of embryos and delayed returns also may be seen.

Diagnosis

The diagnosis is determined by the clinical picture and isolation of the organism, which is easy to grow in the laboratory. Erysipelas should be considered in depressed animals with high temperatures. The development of diamond-shaped skin lesions is considered pathognomonic.

Treatment

Penicillin is the therapeutic agent of choice. Affected animals and all animals in direct contact should receive injectable penicillin. Mass medication of entire

populations also is a very effective method of prevention and can be used in acute outbreaks.

Although vaccination is not entirely effective in preventing the disease, it provides a worthwhile means of control when used with other good management practices. Vaccination should be applied in young swine at about 6 to 8 weeks of age when there is no interference with maternal immunity and again before breeding. Boars also should be vaccinated. Commercially available vaccines include killed vaccines and oral live vaccines given at the end of the nursery stage. If disease breakdowns occur despite vaccination, it is likely that the level of contamination in the environment is high, and proper hygiene measures will have to be implemented accordingly.

EPERYTHROZOONOSIS

Eperythrozoonosis is caused by the rickettsial organism *Eperythrozoon suis*. *E. suis* attaches to the surface of red blood cells and destroys them, causing anemia and icterus.[8]

Epidemiology and Clinical Signs

Infection can be transmitted to pigs by bites from lice *(Haematopinus suis),* infected needles, and ingestion of blood and infected secretions. Infection in utero has been described but is not considered a venereal disease. The disease is very difficult to replicate in healthy pigs under experimental conditions. Stress and compromised immune status may predispose pigs to clinical illness.

Pigs of any age may be affected by *E. suis* infection. Abortion, stillbirths, weak-born piglets, and poor conception rates have been described as reproductive problems in the sow. High fever also can be seen. Young piglets are particularly predisposed to infection. Various degrees of unthriftiness, anemia, and jaundice can be present.[8]

Diagnosis

The most important clinical signs for diagnosis are apathy and fever with temperatures higher than 40° C. Anemia, watery blood, icterus of the carcasses, enlargement of liver and spleen, and presence of fluid in all cavities, with reddening of the bone marrow, are other relevant findings.

The diagnosis is confirmed by demonstrating the presence of organisms on erythrocytes in blood smears stained by Giemsa or acridine orange, particularly at onset of clinical signs.[9] Antibodies can be detected by indirect hemagglutination and indirect immunofluorescence testing; recently, ELISAs using whole organisms also have been described.

Treatment and Control

Administration of tetracyclines by injection in a dose of 20 to 30 mg/kg of body mass is the treatment of choice. Symptomatic treatment such as with iron (200 mg iron/dextran/piglet) also may be required.

Control programs should include ectoparasite management and insect reduction protocols. Management practices directed at preventing exposure to blood of infected animals (i.e., changing needles during vaccination) also will contribute to decreasing transmission. Vaccines against *E. suis* are not available.

ENDOMETRITIS AND THE VAGINAL DISCHARGE COMPLEX

Diseases of the urogenital tract, often referred to collectively as the **vaginal discharge complex**, contribute to poor farrowing rates mainly through the associated increase in regular and irregular returns and decrease in productivity in general. These diseases often are undiagnosed, and up to 25% of herds may have an unrecognized problem with this disease.[10] Individual cases of vulvar discharge rarely represent a major concern. By contrast, if 5% to 10% or more of a breeding group show discharge, then the problem warrants attention.[11] Urogenital diseases are the result of a complex of social, environmental, hormonal, and stress factors that contribute to imbalance of the normal microflora of the urogenital tract, enabling commensal organisms to become pathogens.

Presence of a discharge after service does not necessarily mean failure of the pregnancy but may indicate presence of infection. Discharges may originate from the rectum, vulva, vagina, cervix, and uterus. Discharges also can arise from infection in the kidneys (pyelonephritis) or the bladder (cystitis). The main organisms associated with endometritis and vaginal discharges are opportunistic invaders. The most common organisms include *Escherichia coli, Chlamydia* spp., *Eubacterium suis, Streptococcus suis, Streptococcus equisimilis, Proteus* spp., *Staphylococcus aureus, Staphylococcus hyicus, Pasteurella multocida,* and *Pseudomonas* spp. Bacteriologic tests usually yield mixed cultures, and occasionally only one species may predominate.

Normal discharges are common within 3 to 4 days of farrowing. The sow appears healthy, the udder is normal, and no treatment is needed. Normal discharges also can be seen at mating and up to 5 days after service. A discharge should be considered abnormal if present after 5 days of lactation, between 14 and 21 days after service, and any time during pregnancy. Breeding herds in which vaginal discharges are recorded in greater than 2% are considered to be problem herds.[12]

Predisposing factors for endometritis and the vaginal discharge complex include a high number of older sows in the herd, short lactation lengths (less than 18 days), timing of mating toward the end of the estrus period, nonhygienic conditions during breeding, and housing of the gilts, sows, and boars (natural insemination) in wet, dirty crates and pens. Endometritis may take several forms. Sows usually are clinically normal. Visible changes can range from a pus-filled uterine horn to a slightly hyperemic uterine mucosal surface, or grossly visible changes in the uterus may not be apparent. Endometritis usually results in embryonic death and termination of pregnancy. The typical outcome in long-term cases of endometritis is chronic “recycling” secondary to impair-

ment of mechanisms for embryonic implantation.[10] Endometritis also can occur in nonpregnant animals exposed to environmental stresses.

Diagnosis is done by regular observation of the vulva, particularly 14 to 21 days after mating. Bacteriologic examinations also can be done; however, they are of little help because a wide range of organisms considered to be part of the normal flora may be isolated. Examination of the reproductive tract at slaughter may be beneficial in order to evaluate the entire urogenital tract, as well as to obtain samples for histopathologic study and culture. This procedure may not be practical, however.

Treatment of endometritis can be frustrating. Response to antibiotics may be very limited, and in most cases, animals will not fully recover their reproductive potential. Antibiotics commonly used are injectables or feed-grade antibiotics. Injectable antibiotics should be administered for 3 days (penicillin) or every 2 to 3 days (long-acting tetracycline). Feed-grade antibiotics usually consist of tetracyclines administered at 400 g/ton. Because of the overall poor response to systemic treatments, however, culling of the animals is the most cost-effective solution.

Hygiene in breeding and gestation barns is by far the most important means of controlling vaginal discharges.[12] Pens and crates should be designed to allow easy clean-up. Manure should be removed regularly, and crates should be kept clean and dry. Ventilation is important to enhance drying of the breeding and gestation facilities. At breeding, the perineal area should be cleansed to prevent infections. Special attention also seems warranted to maintaining a clean vulvar area from weaning to 14 days after service. Good hygiene during farrowing is important as well. For assisting at farrowings, technicians should wear clean plastic sleeves and should clean the perineal area before the birth canal is entered.

It is recommended to keep records of occurrence of discharges in sows and to correlate these data with estrus and parturition in order to identify whether a herd problem exists or whether the discharges are occasional.

MISCELLANEOUS INFECTIOUS CONDITIONS

Chlamydial Infection

Chlamydia spp. have been described in swine as agents responsible for causing arthritis, pneumonia, pleurisy, orchitis, uterine infections, and abortions.[13] At least three species are involved: *Chlamydia psittaci*, *Chlamydia trachomatis,* and *Chlamydia pecorum*. *C. psittaci* commonly is isolated from immunosuppressed animals infected with porcine reproductive and respiratory syndrome virus (PRRSV), and in general, it is considered an opportunistic invader. *C. psittaci* also has been identified in a wide range of domestic animals and birds. Outside the host, *Chlamydia* organisms are very resistant to drying and may survive months in that state.[14]

Many chlamydial infections are clinically inapparent, but respiratory and systemic infections may have specific manifestations. Many reports deal with genital tract infections and disturbances in reproduction. In the boar, infection is associated with orchitis, epididymitis, and urethritis, whereas infections in gilts and sows have resulted in late-term abortions and the birth of dead or weak piglets.[13] Infection occurs by inhalation and ingestion of contaminated feeds or through venereal contact. Lesions include enlarged bronchial lymph nodes and raised areas of gray or red consolidation in the lung. Laboratory diagnosis is done by demonstrating the agent in smears of affected tissue or by serologic testing (complement fixation test), immunofluorescence testing, electron microscopy, polymerase chain reaction (PCR) assay, and culture.

Tetracyclines are the drugs of choice, but the organism is not eliminated by the treatment. Vaccines for swine chlamydiosis are not available. Control should include the elimination of other immunosuppressive factors or diseases, proper hygiene, and prevention of exposure to other mammalian species, poultry or poultry droppings, or contaminated feed.

Actinobacillus Species

Various species of *Actinobacillus* have been isolated in cases of reproductive failure. *Actinobacillus ross, Actinobacillus equuli,* and *Actinobacillus suis* have been reported in cases of metritis, abortion, low number of viable pigs at birth, and decreased litter weights.[15,16] *A. ross* is considered part of the normal flora of the porcine vagina, and infection of the uterus may take place during breeding.[16] *A. suis* is considered part of the normal upper respiratory tract flora, and *A. equuli* infection has been associated with direct contact with horses.[15] *Actinobacillus* organisms frequently are isolated in reproductive disorders and fetal tissues. Affected fetuses also may be found to have microscopic lesions in the lungs characteristic of bacterial pneumonia.

Actinobacillus organisms can be readily isolated in the laboratory using standard procedures. Treatment is restricted to therapeutic protocols with variable results. Penicillin has been used for the treatment of *A. suis* infections. Autogenous bacterins also have been used, although their efficacy is doubtful.

Streptococcal Species

Various species of streptococci have been isolated in cases of reproductive failure; however, their significance remains unclear. The species more commonly recovered are *S. equisimilis* and *S. suis*. It is known that these organisms are part of the normal flora present in the vagina and semen. Therefore, their association with reproductive problems may be as secondary pathogens. Nevertheless, streptococci have been isolated in cases of abortion, vaginal discharge, stillbirth, and postparturient agalactia.[17]

Diagnosis is done by culture using standard laboratory procedures. Recommended treatment is administration of an effective antibiotic given intramuscularly. Bacterins are available; however, their efficacy to control reproductive disorders is doubtful. Because *Streptococcus* spp. are considered part of the normal vaginal flora, hygienic

procedures during breeding and parturition need to be practiced.

Toxoplasmosis

Toxoplasma gondii has been isolated in pigs in cases of abortion, stillbirths, premature farrowings, and low-viability piglets at birth.[18] *T. gondii* infection in swine is very uncommon, and for the most part infections are asymptomatic. Cats are the natural reservoir for this protozoan, and it is shed in feces. Transplacental infection can occur; *T. gondii* is a zoonotic agent and poses a risk to pregnant women.

Diagnosis should be directed at identification of cysts in affected tissues. Treatment for toxoplasmosis is not available, and control should be based on proper cat and rodent control programs and proper disposal of aborted tissues. *Toxoplasma* infection is mainly a concern for food safety programs.

Mycotoxicosis

Mycotoxins are secondary metabolites of mold growth in grains and forages. In swine, the most common sources for mycotoxins are corn, wheat, milo, cottonseed, and sorghum. Fungal and plant stressors such as drought, high ambient temperatures, insect damage, and mechanical harvest may predispose crop plants to infestation by toxigenic fungi, with subsequent mycotoxin production.[19] Conditions such as high relative humidity (greater than 70%) and high grain moisture content (23%) favor the growth of *Fusarium* spp.[19]

Two molds are associated with reproductive disorders in swine: *Fusarium graminearum (Fusarium roseum)* and *Claviceps purpurea* (ergot). *F. graminearum* produces zearalenone, which is an estrogenic mycotoxin. Because of its estrogenic activity it causes hypertrophy of the uterus and cornification of the vaginal epithelium.[19] Clinical signs will vary with dose of exposure and age of the affected animal. In prepubertal gilts, concentrations as low as 1 to 5 ppm in the ration will cause vulvovaginitis, edema of the vulva, and early mammary gland development. In mature cycling sows, dietary concentrations of 3 to 10 ppm can induce anestrus if zearalenone is consumed during the middle portion of the estrous cycle. Abortions are unlikely because of zearalenone's luteotropic activity. Anestrus may persist long after exposure to zearalenone has stopped. Reduction in litter size also may be seen. Female piglets may have enlarged, edematous vulvas and swollen teats. Young boars may exhibit decreased libido and reduced testicular size after consuming high levels (200 ppm) of contaminated feed; however, these changes are not observed in mature boars. Diagnosis of zearalenone intoxication usually is made by observing clinical signs of hyperestrogenism in immature females. Feed analysis is available, but it is time-consuming and commonly yields false negative results.

Ergot is a parasitic fungus that affects cereal grains, especially rye, oats, and wheat. The fungus produces alkaloids that cause gangrene and reproductive interference.[19] Ergot alkaloids affect reproduction indirectly by causing agalactia. Pregnant gilts that ingest feed contaminated with the fungus may subsequently give birth to piglets that exhibit low birth weight, low survival rate, and poor weight gains. Ergot in rations of pregnant sows is not generally a cause of abortion, and swine exposed to ergot during late gestation routinely suffer agalactia but rarely abortion. If the clinical signs suggest ergotism, grains should be examined for the presence of significant amounts of ergot sclerotia. Contaminated feed should be removed.

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CHAPTER 107

Viral Causes of Infertility and Abortion in Swine

MONTSERRAT TORREMORRELL

Losses due to reproductive failure are high in today's swine industry. Many factors are involved in causing reproductive failure, including infectious agents, such as bacteria, viruses, protozoa, and fungi and noninfectious causes, such as nutrition, genetics, environment, management practices, and husbandry procedures. This chapter reviews infectious causes of reproductive failure attributable to viruses.

Several viruses have the potential to cause infertility and abortion of swine. The viruses described in this chapter are considered responsible for a majority of the infectious reproductive disorders seen in today's high-technology swine industry. The viruses commonly associated with episodes of infertility and abortion in swine are porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), pseudorabies virus (PRV) (the agent of Aujeszky's disease), classical swine fever virus (CSFV) or hog cholera virus (HCV), porcine enterovirus (PEV), and encephalomyocarditis virus (EMC). Other viruses may be responsible for reproductive failure, but these pathogens are considered sporadic, with a more regional distribution. and typically causing isolated cases, such as porcine cytomegalovirus (PCMV) and blue eye disease virus (BEDV).

Viruses have the ability to cross the placenta and infect and kill the conceptus. Clinical signs will depend on the stage of gestation when the animals become infected, the pathogenetic mechanisms of infection for the virus, and the degree of virulence of the viral strains.

Gestation in pigs lasts about 114 days. In the first 2 weeks of life, the conceptuses lie free in the uterine lumen. Damage to them during this stage is almost always fatal.[1] Implantation begins around day 12 of gestation but is not completed until day 18 to 24 of gestation. Death of the conceptus before implantation will result in resorption of the conceptus, and the sow will undergo a regular return to estrus at approximately 21 days. Death of the embryo before calcification, between approximately 14 and 35 days of gestation, will result in an irregular return to estrus after 24 to 28 days. Complete resorption of the embryos or an abortion may occur. Sows usually return to estrus between 2 and 10 days after loss of pregnancy.

Fetal death after day 35 typically is followed by mummification of the fetus, rather than by pregnancy termination, because the mineral content of the fetus prevents complete absorption. Mummified fetuses are characterized by discoloration and resorption of fluids and soft tissues. If two or more fetuses survive, normal gestation and parturition can still take place.

At about day 70 of gestation, fetuses begin to develop immune competency. The epitheliomaternal placenta normally does not allow transfer of maternal antibodies to fetuses. Infection before 70 days by certain viruses may result in immunotolerance for the surviving fetuses. Immunotolerant-born piglets may be viremic at birth, and the virus is not recognized by the pig's immune system.

Infection after 70 days of gestation may result in a successful immune response by the fetus, with the production of antibodies. Although a fetus may successfully produce antibodies, it may still succumb to the virus and be stillborn.

Abortion refers to the delivery of an immature fetus, either live or dead, before the completion of the gestation period as a result of the failure of the mechanisms that control pregnancy. Abortions typically result from failure of some maternal system, rather than the effects of the

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viruses on the fetuses. These system failures and the resultant abortions usually are sequelae of systemic effects of viruses on the dam, such as high fever or viremia.

A **stillbirth** is a fetus that has matured fully in utero but is born dead. Stillborn piglets look normal, but their lungs do not float in water (respiration never occurred before death). Certain viruses have tropism for fetuses at the end of the gestational stage. Stillbirths also may be caused by noninfectious agents or conditions and are known as **intrapartum deaths.**[1]

If less than the entire litter is infected transplacentally, the virus may spread within the uterus and infect littermates at different gestational ages, resulting in a variety of responses within the litter, including reduced litter size, mummification of fetuses, and stillbirths.

Viruses associated with reproductive failure also may cause increased neonatal mortality. **Neonatal death** refers to that occurring primarily within the first 7 days of life. Death usually is due to events that take place during late gestation, or shortly after parturition, or weak piglets infected late in gestation may die soon after birth. In production records, neonatal death usually is captured within the preweaning mortality parameter.

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

Porcine reproductive and respiratory syndrome virus (PRRSV) is a fairly new virus first reported clinically in the late 1980s and isolated in Europe in 1991, and in the United States in 1992.[2] PRRSV has become the most important virus affecting the swine industry, with estimated annual costs of U.S. $570 million in the United States alone.[3]

Epidemiology and Clinical Signs

PRRSV characteristically causes both reproductive and respiratory clinical signs.[2] Reproductive clinical signs include late-term abortions, premature farrowings, weak-born piglets, and increased preweaning mortality rate. Respiratory clinical signs are due to the presence of interstitial pneumonia in nursery and finishing pigs, which can be complicated by secondary infections. Two main genotypes are recognized: European (or Lelystad virus) and North American. Within each genotype, significant strain variation exists. Differences in strain variation may influence virulence, pathogenesis, and clinical signs.

PRRSV is widely distributed throughout the world. Only few countries are considered free of the disease. Infected countries have widespread prevalence. PRRSV is readily transmitted by contact exposure of secretions of infected animals such as serum, saliva, milk, urine, and feces. PRRSV also is transmitted by semen, and this route of infection has gained greater importance since the increased use of artificial insemination and the establishment of central distribution boar studs. Infected animals can be viremic for prolonged periods of time. Duration of viremia will depend on the age of the animal, with longer periods of viremia in younger pigs than in older animals. After viremia has ceased, virus still persists in the tissues. Different studies show that such persistence may be detected in tissues for more than 100 days. Contact transmission has proved limited, however, in general lasting for up to 80 days after infection, although exceptions may exist.

PRRSV can be readily transmitted by contact with infected surfaces and through iatrogenic transmission by needles or materials that facilitate body fluid exchange.

Transport vehicles not properly cleaned and dried, contaminated fomites, exchange of material between infected and noninfected herds, and failure of farm personnel to follow biosecurity measures are considered important sources of infection between herds.[4–6] Insects and aerosol transmission also have been described as possible sources of infection, but their relevance is still unclear or limited to very specific regions. PRRSV is an RNA enveloped virus that survives well in cold and wet environments, and its infectivity decreases as infected surfaces become dry.

Diagnosis

Late-term abortions, premature farrowings and significant increase in preweaning mortality rate are very suggestive of PRRSV infection.[2] Acutely infected young piglets will be viremic for prolonged periods, up to 35 days. Blood samples and infected tissue specimens to detect presence of virus can be tested by virus isolation or polymerase chain reaction (PCR) assay. Several serologic tests that are based on enzyme-linked immunosorbent assay (ELISA) techniques are available commercially. Immunofluorescence assay (IFA) and IPMA tests also are available and are deemed more specific but less sensitive. Serologic tests measure exposure to the virus but not protection. When seronegative populations are monitored using these tests, serologic reactors may be found. In such instances, first performing a test with high sensitivity is recommended, followed by a more specific testing of the reacting samples. In the United States, the combination of ELISA followed by IFA has become the standard diagnostic laboratory procedure.

Infected piglets will be found to have microscopic lesions associated with PRRSV infection. In investigating a reproductive PRRSV infection, samples from weak-born piglets will constitute a good source of virus. Samples from mummified fetuses may yield false negative results because the virus may have become degraded.

Prevention, Control, and Eradication

Recent years have seen considerable advances in the area of prevention and control of PRRSV disease. Many new management techniques provide a means of control and even eradication of most PRRSV infections on swine farms. Control of PRRSV disease is directed at limiting damage from the viral infection; this strategy it is based on management of the replacement animals and prevention of reinfection by new viral strains.

A majority of new infections affecting commercial systems in the United States are considered to be a result of lateral transmission and not due to introduction of infected pigs or semen.[6] Area spread from neighboring units, transport of pigs in PRRSV-infected equipment, lack

of compliance with biosecurity protocols, and even potential introduction by means of insects[5–8] are considered risks for PRRSV introduction into herds. Biosecurity protocols need to be properly thought out and implemented. Special effort should be made in selecting isolated areas for establishing new units and in reviewing all of the procedures that involve movement of farm inputs and outputs——transport of pigs, supplies and materials, feed, water, and personnel; removal of manure; and reclaims. Because of the nature of the virus and its excellent survival in cold and wet conditions,[4] procedures must ensure the cleaning and drying of all equipment and material used at the farm. In particular, all equipment used for transport of pigs should be properly cleaned and completely dry.[5,9] All units also should have biosecurity measures in place to prevent infestation by pests such as rodents, insects, and birds.

Considerable advances in the control of PRRSV disease have been achieved during the last several years; however, control measures remain one of the most frustrating items for practitioners. The central component of PRRSV disease control is the reduction of the spread of the virus within the breeding herd, thereby preventing the infection of offspring before weaning.[10] The perpetuation of infection in chronically infected herds may potentially be due to the presence of subpopulations of animals of differing immune status and virus activity.[11] A step to prevent the development of such subpopulations has been the introduction of replacement animals that are previously exposed and immune to PRRSV infection before introduction in the PRRSV-positive herds.[12] The consistent acclimatization of incoming breeding stock to PRRSV results in the stabilization of clinical signs of disease, the improvement of production parameters, and the production of PRRSV-negative piglets at weaning; therefore, gilt introduction remains the key to PRRSV control.[13] Different methods for gilt acclimatization exist, and in general it is accepted that early exposure (at 2 to 4 months of age) will result in homologous protection of the exposed animals and introduction of the replacement animals at a time when shedding has stopped. A major challenge, however, remains the exposure method that will consistently induce PRRSV infection.

Control measures in the weaned pig population should be directed at minimizing the commingling of different pig sources at weaning, streamlining flows, strict implementation of all-in–all-out animal flow, implementation of nursery partial depopulations, and controlling other concurrent infections, mostly bacterial (e.g., *Haemophilus parasuis, Streptococcus suis, Mycoplasma hyopneumoniae*) and viral (i.e., swine influenza virus) aggravated by the PRRSV infection. Appropriate vaccination and medication protocols need to be determined for the individual infections. Finally, if the suckling piglet population is acutely infected, a series of management strategies directed at limiting the movement of piglets between litters in the initial 24 hours of life, humanely destroying chronically infected offspring before weaning, and maintaining strict all-in–all-out animal flow for the nursery should be helpful.[14]

Eradication of PRRSV is possible. Spontaneous elimination has been described in small-herd units.[15] Consistent PRRSV eradication was not possible until recently, however.[16,17] Total depopulation-repopulation, partial depopulation, segregated early weaning, testing and removal, and herd closure are among the most common strategies. The success of PRRSV elimination in the breeding herd resides in the introduction of seronegative nonexposed replacement animals into the breeding herd at a time when virus is no longer present.

Total herd depopulation-repopulation is a very successful technique, although it is costly and may be justifiable only if the elimination of other concurrent diseases is desired. Partial depopulation is indicated for the elimination of the virus from growing pigs when shedding from the breeding population has completely stopped. This technique alone has proved to be enough to eliminate the virus in small size farms.[18] PRRSV elimination through the herd closure technique has gained considerable popularity in the United States.[17] The principle of this technique is based in the fact that PRRSV infection tends to die out in an immune population over time. This strategy mimics the principles followed for eradication of transmissible gastroenteritis (TGE) in pigs, which ensure that all animals are exposed to the virus and that replacement animals that could perpetuate virus replication are not introduced during that time. In the case of PRRSV, longer periods of herd closure with no introduction of new replacement animals are required. Animals recovered from the infection will eliminate the virus from their tissues, although this will require prolonged periods (about 6 months). Introduction of seronegative replacement animals will be followed by attrition or scheduled culling of the previously infected animals. PRRSV elimination through the test and removal technique has also been used with successful results.[16] Elimination of PRRSV by testing and removal consists of blood testing the entire breeding herd, identifying PRRSV-infected animals using tests for both antibody and virus, and removing seropositive animals from the farm.

Several studies have established that vaccination against PRRSV can result in protective immunity.[3] Several commercial vaccine products are available today. Both attenuated and inactivated products are available on the various continents. In general, it is accepted that attenuated modified live virus vaccines induce a more efficacious immune response, although concerns regarding safety have been raised for some of the products. Inactivated vaccines also are available, but in general they are considered less efficacious when used in seronegative animals.

When used in the field, vaccines have met with various degrees of success in different countries. Differences may be due to differences in the commercial products available and how these products are utilized, and to differences related to presence of viral strains in different regions in which cross-protection is believed to be very limited. Also, induction of protective immunity among genetically different PRRSV strains, although it is possible, is very limited and difficult to assess. In addition, field reports that document the transmission of the vaccine virus and its reversion to the vaccine virus virulent form also can be found in the literature. Some vaccine virus strains may behave very similarly to field PRRSV strains

in relation to persistence, transmission to virus-naive pigs, crossing of the placenta to cause congenital infection, shedding in semen, and length of time required to induce protective immunity. Therefore, issues regarding the safety and efficacy of the live virus vaccine products remain unresolved. Research to provide safer and more efficacious products is in progress.

PORCINE PARVOVIRUS

Porcine parvovirus (PPV) causes reproductive disorders characterized by resorption of infected fetuses, increased number of fetal mummies, and reduced litter size, usually in the absence of obvious maternal clinical signs.[19] Traditionally, clinical signs of parvovirus infection are seen in gilts or sows of low parity. There is no evidence that infection of swine at times other than during gestation is of any clinical or economic significance.

Epidemiology and Clinical Signs

Porcine parvovirus is ubiquitous throughout the world. Infection is considered enzootic in most swine herds. Contaminated premises probably are major reservoirs for PPV. PPV is a nonenveloped virus resistant to most common disinfectants. In an established seropositive farm, gilts usually become infected after maternal antibodies have disappeared. In many instances, maternal antibodies may last up to 6 months. Gilts usually become infected by exposure to feces through the oral-fecal route as maternal antibodies wane. In order to prevent reproductive clinical signs, gilts must become immune before the first breeding, which usually occurs at about 6 to 7 months of age. In addition, acutely infected boars also may play a significant role in disseminating PPV. Semen also may become infected through external contamination with feces at collection for artificial insemination practices.

Dams may return to estrus, fail to farrow despite being anestrous, farrow few pigs per litter, or farrow a large proportion of mummified fetuses. All of these clinical scenarios can reflect occurrence of embryonic or fetal death, or both. PPV infection in early gestation will result in the resorption of fetuses and irregular return to estrus. Dams are susceptible to PPV infection during about the first half of gestation. Infection after 30 days of gestation will cause fetal death, resulting in mummification. PPV usually does not infect all fetuses at once. Usually fetuses will become infected sequentially, resulting in mummies of different sizes at birth. Fetuses infected after 70 days of gestation will be able to mount an immune response and usually will survive without obvious clinical effects in utero. Abortions are rare. Reduced litter size with multiple mummies and healthy piglets may be observed at birth. In the boar, evidence is lacking for an effect of PPV on fertility or libido.

Diagnosis

PPV infection should be considered in a differential diagnosis of reproductive failure of swine whenever there is evidence of embryonic or fetal death, or both. Litters with multiple-size mummies observed in first-parity gilts usually are characteristic of PPV infection. Abortions are rarely seen.

Fetal mummies infected before 70 days of gestation (size less than 16 cm) will carry the virus in multiple tissues. Mummy emacerate can be tested for the presence of virus using the hemagglutination (HA) test. Mummies infected after 70 days of gestation will have antibodies that can be assessed in thoracic fluid by the hemagglutination inhibition (IHA) test.

Serologic status of gilts and sows can be assessed using the IHA test. Because PPV is ubiquitous, the presence of antibody in a single sample is meaningless. Paired serum samples indicating a significant rise in antibody level is required to prove infection. In order to detect seroconversion on a population basis, a serum profile with the collection of 10 samples from multiple growing-age gilts and breeding-age gilts is recommended to determine time of seroconversion for the gilts. Titers induced by natural infection tend to be very high and confer lifelong protection.

Treatment and Control

Control of PPV infection is centered on ensuring immune status of the animals before breeding. Gilts should be either naturally infected with PPV or vaccinated for PPV before they are bred. Feedback exposure to feces from older animals or contact exposure between seropositive and seronegative animals as a measure to induce natural immunity in gilts is a common measure. A commercial killed vaccine also is available. Time of vaccination is critical, however, because maternal antibodies are long-lasting and may interfere with vaccine efficacy. Vaccination requires at least two injections 2 to 5 weeks apart before initiating breeding of the gilts. Subsequent vaccine administration in older-parity animals is recommended before each breeding. Vaccine administration also is recommended in boars.

PSEUDORABIES VIRUS

Pseudorabies virus (PRV), the agent of Aujeszky's disease, is a herpesvirus with the ability to establish latent infections. PRV causes reproductive failure characterized by abortion, embryonic death resulting in return to estrus, mummification, and stillbirths.[20] PRV also causes CNS signs in younger pigs and respiratory signs in growing and adult swine.

Epidemiology and Clinical Signs

The pig is the only natural host of PRV, which accounts for its ability to be subclinically and latently infected. Latently infected carrier animals can sporadically shed the virus throughout their lives. Infection in other farm animals such as cattle, sheep, goats, dogs, and cats is fatal, and herds located near pig farms often act as sentinels for the infection.

Transmission of the virus is by direct contact with infected animals, primarily other pigs. The virus is shed

in nasal and oral secretions and also can be transmitted transplacentally, by contact with the vaginal mucosa, and through semen and milk. Farm-to-farm movement of animals that are shedding the virus probably is the more common mode of spread of infections. Movement of contaminated feed, bedding, trucks and other vehicles, and people also plays an important role. Aerosol transmission also is believed to be possible under favorable environmental conditions of temperature and humidity. Exposure to infected feral pigs also can be an important source of infection. PRV has relatively low survivability in the environment and is susceptible to disinfectants and to drying, especially in the presence of direct sunlight.

Primary infection occurring as an outbreak of disease in a seronegative unprotected herd can be a devastating event. Infection will spread quickly throughout the entire herd. Clinical signs will vary depending on the virus virulence, the infectious dose, level of protection already existing in the herd (if already vaccinated or infected), and, more important, depending the age of the swine affected. Younger piglets are the most severely affected. Suckling piglets will exhibit CNS signs resulting in very high preweaning mortality. Weaning-age pigs also will exhibit nervous signs; usually these are less severe, but respiratory signs also may be present. Infected pigs typically develop high fevers. In grower-finisher pigs, respiratory signs have become the hallmark of PRV infection. Central nervous system signs may occur but only sporadically. Growth retardation also is seen. Adult swine may exhibit fever, anorexia, depression, and respiratory clinical signs, with the primary sign being reproductive failure if sows are pregnant. Infection in the first trimester of gestation may result in fetus resorption and return to estrus. Reproductive failure in the second and third trimesters usually is manifested as abortion or, if infection occurs close to term, as stillbirths or birth of weak piglets. PRV can cross the placenta and cause placentitis, resulting in abortion at any stage of gestation.

Diagnosis

Classic clinical signs often will lead to a presumptive diagnosis that often is supported by observation of gross lesions of focal hepatic, pulmonary, and splenic necrosis and necrotic tonsillitis in neonatal pigs. Additional microscopic lesions are seen primarily in the nervous system and include nonsuppurative meningoencephalitis, ganglioneuritis, and perivascular cuffing by mononuclear cells.

Several serologic tests are available. In most cases, positive test results indicate exposure to the virus and not necessarily causation. The most widely used tests are serum neutralization, latex agglutination, ELISA, and differential ELISAs that allow differentiation between antibodies induced by a gene-deleted vaccine and antibodies induced by natural infection. The infection also is confirmed by direct detection of the virus by immunofluorescent antibody test or isolation of the virus from fetal or neonatal tonsils, brain, spleen, or lung.

Treatment (Vaccination) and Control

Modified live virus, inactivated, and gene-deleted vaccines have been developed for the control of PRV disease and are available in most countries in which PRV disease is endemic. These vaccines have proved to be quite effective in reducing or preventing clinical signs and thus have reduced the economic impact of the disease. Vaccination will not prevent the infection, however.

Because of the advantages of using a differential ELISA diagnostic test, gene-deleted vaccines are preferred. Animals vaccinated with a gene-deleted vaccine will lack antibody against the specific glycoprotein encoded by the deleted gene, allowing a vaccinated pig to be differentiated from an infected one. In addition, these vaccines become extremely useful in the initial stages of a control and eradication program.

Most major swine-producing countries worldwide have undertaken area control and eradication programs. Recently, the United States has completed the eradication of the virus, and all states are considered Stage V, with surveillance at a maintenance level. Vaccination is then prohibited.

CLASSICAL SWINE FEVER

Classical swine fever is caused by the pestivirus classical swine fever virus (CSFV), also referred to as hog cholera virus (HCV). This disease still has a worldwide distribution, but gradually more countries are succeeding in the eradication of the virus. The United States became CSF free in the late 1970s. The virus is still an important pathogen in some parts of the world.

The pig is the natural host and is the significant source of spread of CSFV.[21] Direct contact between infected and susceptible pigs is the principal means of viral transmission. CSFV is excreted with oronasal and lacrimal secretions, urine, and feces. Chronically infected pigs shed the virus continuously or intermittently until death. Mechanical vectors contribute to the spread of CSFV between premises (equipment, trucks), but virus also may be mechanically transmitted by pets, birds, and arthropods. CSFV survives in frozen meat, and garbage feeding to pigs has resulted in clinical outbreaks of CSFV disease.

Gross lesions range from none in peracute infections to swelling, edema, and multiple hemorrhages in the lymph nodes and kidneys. Petechial and ecchymotic hemorrhages in the kidneys are common, and infarction of the spleen is considered very characteristic for CSFV infection. Infarction and hemorrhage also occur in the lungs. Encephalitis with perivascular cuffing may be seen microscopically.

Congenital CSFV infection can result in abortion, fetal mummification, stillbirth, malformations, and the birth of weak pigs with tremors, or of healthy-looking yet infected piglets. Among piglets infected in utero, skin hemorrhages are common, and the neonatal mortality rate is high. Piglets infected in utero may be persistently infected at birth and appear to be immunotolerant to the virus. Piglets can recover from an in utero–acquired CSFV infection, however.

CSFV infection may be confused on clinical and pathological grounds with several other viral or bacterial infections; therefore, it is recommended that clinical diagnosis be confirmed by the laboratory. It is virtually impossible to make a clinical diagnosis in cases of subacute, chronic, or late-onset classical swine fever because of the wide variability of clinical signs and pathologic lesions. Laboratory diagnosis is based on detection of viral antigen, isolation of virus, or demonstration of viral antibody. The direct fluorescent antibody test performed on frozen tissue sections is the method of choice for detecting viral antigen. Serologic tests also are available but cannot differentiate between vaccine- or infection-induced antibodies.

In countries in which CSFV is enzootic, vaccination is often practiced, and in some countries vaccination is supplementary to the killing of infected herds. Countries free of CSFV have stringent measures directed at preventing the introduction of the virus. Countries ban the import of live pigs, pork, and nontreated pork products from countries in which CSFV is present.

PORCINE ENTEROVIRUS

Porcine enteroviruses are ubiquitous and found in a majority of pig herds. They belong to the family Picornaviridae and are highly resistant to the rigors of the environment.

Although a majority of infections are asymptomatic, porcine enteroviruses have been associated with a variety of clinical presentations, including polioencephalomyelitis, female reproductive disorders, enteric disease, and pneumonia.[22]

The primary route of transmission is the fecal-oral route, and indirect transmission by fomites is very likely to occur because the viruses are relatively resistant.

Several serotypes of the virus exist. These differ in degree of virulence and clinical manifestations of infection.

Porcine enteroviruses can be associated with stillbirths, mummification of fetuses, embryonic death, and infertility. It is important to differentiate disease due to these organisms from parvovirus infection, because clinical signs are very similar. No remarkable lesions are seen in stillborn or neonatal piglets, although mild focal gliosis and perivascular cuffing in the brain stem have been found occasionally.

Commercial vaccines are not currently available. Controlled exposure, similar to that described for control of parvovirus, is the best method to prevent clinical signs. Diagnosis is confirmed by virus isolation, which is best performed using fetal lung tissue, or by detection of viral antigen by immunofluorescence.

ENCEPHALOMYOCARDITIS VIRUS

Encephalomyocarditis virus (EMCV) is a picornavirus regarded as a rodent virus, although the virus naturally infects a wide range of vertebrate species. Pigs are the domestic animals most susceptible to clinical disease by EMCV infection.[23]

Historically, the virus has been considered the cause of myocardial disease in neonatal pigs. It received considerable attention in the late 1980s and early 1990s as the potential cause of "mystery pig disease," which was later confirmed to be caused by porcine reproductive and respiratory syndrome virus (PRRSV).

The most important sources for swine infection appear to be feed and water contaminated with the virus by rats, other rodents, or infected carcasses. Therefore, it is important to control rodents on pig farms and minimize their contact with pigs either directly or indirectly through contamination of feed or water.

In breeding females, clinical presentation varies, ranging from no obvious illness to severe reproductive problems. Evidence of reproductive failure includes abortions and increased numbers of mummified and stillborn fetuses. Increased neonatal deaths and preweaning mortality also may be observed.

Diagnosis is confirmed by virus isolation from heart tissue of fetuses and young pigs. Alternatively, serum neutralization or IHA tests can be performed on serum or fluids from stillborn pigs or mummified fetuses for detection of antibodies to the virus.

CYTOMEGALOVIRUS

Porcine cytomegalovirus (PCMV) is a herpesvirus that usually induces a clinically silent infection in the adult; in the young pig it causes rhinitis characterized by inclusion bodies and may cause pneumonia.[24] The infection can be fatal in young piglets infected systemically. The virus has worldwide distribution. In susceptible pregnant sows the virus may infect the embryo shortly after implantation, which may lead to embryonic death. PCMV also may cause mummification of fetuses and stillbirths. Neonatal death also may occur; at necropsy, affected pigs exhibit retardation in growth with rhinitis or pneumonia.

Diagnosis requires laboratory support. Serologic testing and viral isolation are difficult to perform. Identification of the virus needs to be confirmed by immunostaining from nasal scrapings.

BLUE EYE DISEASE

Blue eye disease is caused by a paramyxovirus and is characterized clinically by central nervous system abnormalities, corneal opacities, and reproductive failure.[25] Blue eye disease has been reported clinically only in the central part of Mexico, although other members of the Paramyxovirus family have been isolated in swine in other countries. Reproductive failure is manifested as fetal death with return of the sows to estrus, stillbirths, and mummified fetuses. Abortion is not a feature but may be observed in the face of an acute outbreak. In boars the disease may manifest as orchitis and epididymitis.

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CHAPTER 108

Noninfectious Causes of Infertility and Abortion

RODERICK C. TUBBS

ANATOMIC ABNORMALITIES

Anatomic abnormalities are present in about 2% of all newborn pigs. Nonlethal anatomic aberrancies may result in infertility and may account for 10% to 20% of cases of reproductive failure in nonbreeding gilts. Most anatomic abnormalities, except those of the external genitalia, are not apparent in live animals. Because of the frequency of occurrence of anatomic abnormalities, gilts that do not cycle within 30 to 60 days after selection or delivery, or those that do not conceive after the second or third mating, should be culled. Generally, diagnostic investigations of individual gilts are not justified; however, if more than 10% of a group of replacement gilts have infertility problems, a diagnostic investigation, including slaughter checks, is indicated.

CYSTIC OVARIAN DISEASE

Cystic ovarian disease is a common cause of reproductive failure in sows. Cystic ovaries may be present in 10% to 25% of infertile sows. Single cysts are an incidental finding at slaughter and do not affect cyclicity or fertility. Multiple cysts, small or large, may be associated with infertility. Multiple small cysts (10 to 15 mm in diameter) secrete estrogen and occasionally may be associated with anestrus. Multiple large cysts (15 mm to 10 cm) secrete progesterone and commonly are associated with anestrus.

PHYSICAL CONDITIONS THAT INTERFERE WITH REPRODUCTION

Physical problems that potentially interfere with reproduction include injuries of the vulva or vagina, or both; musculoskeletal problems; and diseases of the gastrointestinal and cardiovascular systems.

Trauma

Trauma to the genital tract may result from vulva-biting by aggressive sows in group housing systems, from farrowing injuries, or from breeding injuries. These types of injuries usually impair reproduction by causing adhesions that occlude the reproductive tract, thereby interfering with coitus or gamete transport. They also predispose the affected animal to the development of chronic vaginitis or endometritis. Animals with severe injuries to the lower genital tract or chronic endometritis as a result of those injuries should be culled.

Musculoskeletal System

Lameness due to foot pain, poor conformation, trauma, footrot, or nonseptic laminitis may predispose affected animals to reproductive problems. Gilts may be added to the breeding herd but never mated because they are too lame to mate or because they exhibit weak physical signs of estrus. Pregnant sows may be culled because of foot and leg problems. In some herds, musculoskeletal diseases represent the primary reproductive problems because of their impact on nonproductive sow days, farrowing rate, cull rate, death rate, and, subsequently, overall replacement rate.

Nonseptic laminitis occurs secondary to injury and is characterized by inflammation of the corium, foot pain, and a digital pulse. The front limbs are primarily affected. Damage to the hoof pad or hoof wall may allow penetration by bacteria into the corium, resulting in footrot, subsolar abscesses, or other hoof problems. Traumatic injury to the hooves is more common on the lateral toes, possibly because they bear more weight than the medial toes. Joint or bone infections may occur secondary to trauma as well.

Other musculoskeletal system problems that may interfere with reproduction, especially in gilts, are osteochondrosis and degenerative joint disease. These conditions result from developmental lesions that affect cartilaginous growth plates. Clinical signs range from subtle changes in gait to inability to stand. Such signs usually are noted in the first 4 to 8 months of life. Genetic background, conformation, and floor type are believed to contribute to the condition, although these conditions may develop in any fast-growing animal. Direct correlations between growth and nutrition and osteochondrosis and degenerative joint disease lesions are difficult to establish. The best methods to prevent joint lesions are to provide adequate nutrients, feed intake, and a proper environment and to select breeding stock with good physical conformation from genetic lines with no history of the condition.

Gastrointestinal System

Gastrointestinal problems may contribute to reproductive inefficiency either directly, by interfering with exhibition

of strong physical signs of estrus, or indirectly, by causing death or disorders leading to culling of pregnant sows. Common gastrointestinal problems include gastric ulcers and gastrointestinal accidents such as gastric dilatation, torsion, or volvulus. Clinical presentation with gastric ulcers is characterized by anemia, sudden death, gastrointestinal pain, vomiting, and bloody or dark feces. The definitive lesion seen at necropsy is ulceration of the pars esophagea. Maintenance of feed particle size at 700 to 800 μm or greater and added fiber in gestation diets may be helpful in preventing gastric ulcers.

Gastrointestinal accidents occur sporadically but may be common in some herds. Sows are predisposed to occurrence of gastrointestinal accidents by irregular feeding times, feeding of gruel or whey, and excessive excitement at feeding time. Establishment of a regular feeding time, addition of fiber to the diet, and use of a drop-feeding system that delivers feed to all sows simultaneously are the best methods for prevention.

Cardiovascular System

Cardiac failure is a commonly diagnosed cause of death in some sow herds. Obesity, parturition, heat stress, copulation, fights, and transport predispose affected animals to cardiac failure. A diagnosis of cardiac failure should be made only after other causes of death in adult sows have been ruled out.

TOXINS

Many compounds are potentially toxic to pigs, but few are specific to the reproductive tract and even fewer are likely to come into contact with sows in modern production systems. The discussion here is restricted to mycotoxins, primarily zearalenone, and carbon monoxide toxicity.

Mycotoxins

Mycotoxins are produced by molds that grow in grains such as corn, milo, and wheat under the proper conditions of moisture and oxygen content. Although commonly blamed for reproductive problems, **aflatoxins** have not been shown to have significant reproductive effects in swine.[1] **Ergot alkaloids**, produced by the fungus *Claviceps purpurea,* cause low birth weights, reduced survival, and diminished weight gains in piglets and agalactia in sows but only rarely cause abortion. **Fumonisin**, produced by *Fusarium moniliforme,* is associated primarily with a respiratory syndrome characterized by cyanosis, hydrothorax, and diffuse interstitial and interlobular pulmonary edema. Pregnant sows that recover from the respiratory disease may abort 2 to 3 days later.[1]

Zearalenone, also known as F-2 toxin, is produced by *Fusarium graminearum (Fusarium roseum).* Zearalenone is an estrogenic mycotoxin that is produced under conditions of high relative humidity (higher than 90%) and high moisture content (greater than 25%).[1] The severity of clinical signs caused by zearalenone varies with the level of exposure and age of the animal. Prepubertal gilts may develop vulvovaginitis, characterized by swelling and redness of the vulva, when exposed to as little as 1 to 2 ppm in the ration.[2] Vaginal and rectal prolapses are common sequelae. Removal of the contaminated grain from the diet for 40 days, even after 90 days of exposure to 1.5 to 2 ppm, results in normal sexual maturity and normal subsequent reproductive performance in gilts.[3]

In mature sows, zearalenone is luteotropic; ingestion of 3 to 10 ppm during midcycle maintains luteal function and progesterone secretion and leads to anestrus. Exposure to zearalenone during early pregnancy, particularly during the preimplantation stage (7 to 10 days after mating), may decrease litter size.[4]

The primary effect of zearalenone in boars is preputial enlargement, and no apparent reproductive problems have been recognized in mature boars exposed to levels of up to 200 ppm.[5] In young boars, testis size and libido may be reduced.[6]

Treatment for any of the conditions associated with mycotoxicosis is removal of contaminated grain. Definitive identification of mycotoxins in feed or grain samples may be difficult, because the molds tend to occur in "hot spots" and may have been consumed by the time the sample is taken. Although the clinical signs caused by most mycotoxins may be nebulous and may be difficult to document, vaginal swelling and precocious mammary development in prepubertal gilts can be considered pathognomonic for exposure to zearalenone.

Carbon Monoxide Intoxication

The primary reproductive manifestation of carbon monoxide intoxication is an increase in the number of stillborn pigs. Toxicity occurs when gas-burning space heaters or furnaces are operated improperly in tightly constructed, poorly ventilated farrowing houses. High concentrations of CO (greater than 250 ppm) can result in an increase in number of stillborn pigs, can cause sows to deliver dead pigs within a few hours after entering a farrowing house, or may be associated with the birth of entire litters of stillborn pigs to multiple sows.[7] Suspected CO-induced cases of stillbirths can be confirmed by (1) measuring the amount of CO in the air or (2) measuring the percentage of carboxyhemoglobin concentration in fetal thoracic fluid (concentrations greater than 2% are associated with CO intoxication).[7]

SEASONAL INFERTILITY

The primary noninfectious cause of infertility on most swine farms, other than management practices, is seasonal infertility.[8–11] The domestic pig is a year-round breeder, but its ancestor, the European wild pig, has a distinct seasonal breeding pattern, and domestic pigs have retained at least some of that characteristic as evidenced by the sharp decline in reproductive efficiency during late summer and early autumn on many farms. All measures of reproductive efficiency are affected, including decreased farrowing rates as a result of autumn matings (followed by reduced farrowing rates in December and January), increased wean-to-service interval (WSI), increased numbers of stillbirths and abortions, and fewer

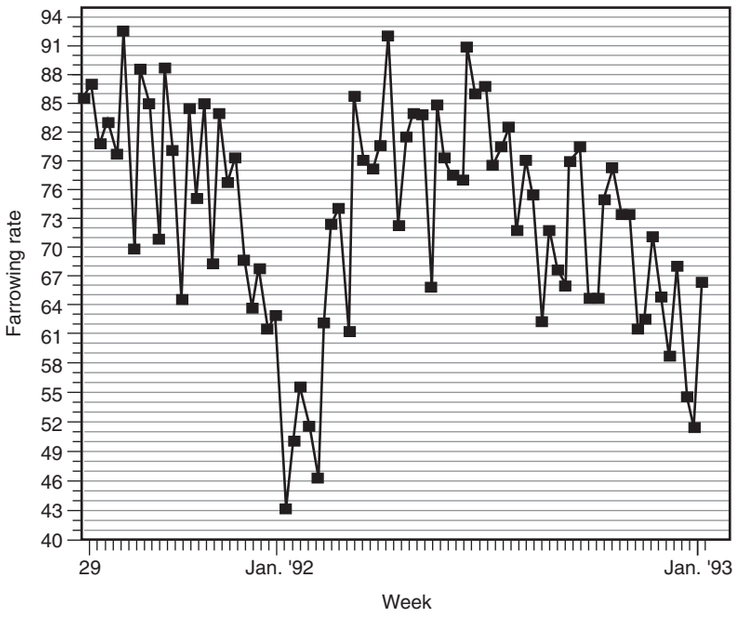

Fig. 108-1 Farrowing rate for a 1000-sow farm during 1992 and 1993. Note depression in farrowing rates during the winter months of each year (corresponding to late summer–early autumn matings). (From Tubbs RC, Zulovich JM: Facility design and housing for swine. *Vet Med* 1995;90:893.)

liveborn pigs. Progesterone concentrations are lower in sows during late summer and early autumn, and this decrease may indicate a propensity for pregnancy loss.[12]

Although high ambient temperatures frequently are blamed for seasonal infertility by producers and veterinarians, the problem probably is not related to heat, or to reduced semen production or libido in boars. The phenomenon of seasonal infertility is more likely to be related to the response of sows to changes in photoperiod. The exact mechanism has not been elucidated, and disagreement exists about whether sows respond to increasing photoperiod before the summer solstice (June 21) or to decreasing photoperiod after the summer solstice. Decreased photoperiod after the summer solstice may signal sows to become less reproductively efficient, possibly to avoid farrowing in the winter. The result is a decrease in farrowing rate due to reduced pregnancy maintenance. The number of sows that return to estrus after mating at regular intervals (18 to 25 days after mating) or irregular intervals (later than 25 days after mating) increases; a relatively greater increase is seen in the number of sows that return to estrus after a prolonged interval. Prolonged WSI in sows and anestrus, especially in gilts, occur as well. The peak time for abortions is around September 21, which falls between the summer solstice (June 21; longest daylight) and the winter solstice (December 21; shortest daylight).[13] Figure 108-1 shows the seasonal infertility pattern observed on a 1000-sow farm in the southeastern United States in 1992 and 1993.[14] Birth weights, weaning weights, and preweaning mortality rate also appear to have seasonal patterns on many farms, but these effects may be related primarily to temperature, rather than to photoperiod.

MANAGEMENT PRACTICES

Most reproductive problems on swine farms are related to the way in which animals are managed. The pivotal point

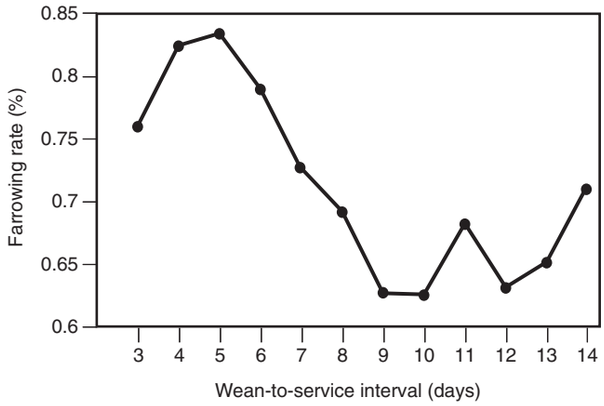

Fig. 108-2 Farrowing rate by wean-to-service interval. Based on records from approximately 51,000 hand matings. (From Tubbs RC: Evaluating management causes of swine reproductive failure. *Vet Med* 1995;90:195.)

of swine breeding programs occurs in the farrowing house, not in the breeding barn. Feed intake during lactation is the key to maintenance of reproductive performance from one litter to the next. Sows must have high levels of nutrient intake to rebreed quickly after weaning. A short WSI is important to achieve high subsequent farrowing rates and litter sizes (Figs. 108-2 and 108-3).[15] High feed intake during lactation, especially during the first 2 weeks of a 3-week lactation, is critical to stimulation of gonadotropin activity and reduction of the WSI.[16] To achieve a 3- to 6-day WSI, the following levels of nutrient intake are recommended during lactation: 16 Mcal of metabolizable energy, 800 to 1000 g of protein, 45 to 50 g of lysine, 40 to 50 g of calcium, and 35 to 40 g of phosphorus.[17]

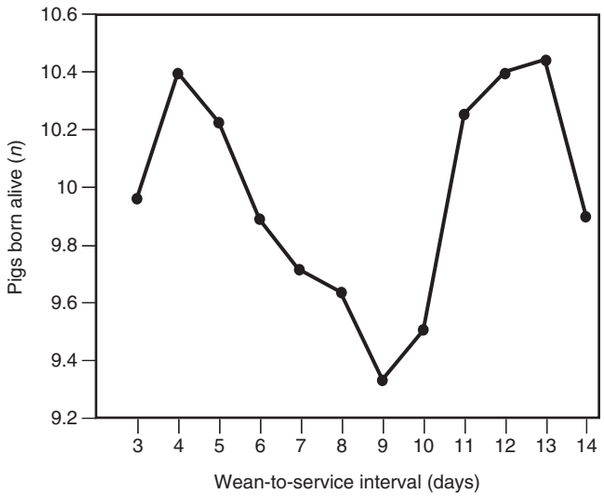

Fig. 108-3 Pigs born alive per litter by wean-to-service interval. Based on records from approximately 51,000 hand matings. (From Tubbs RC: Evaluating management causes of swine reproductive failure. *Vet Med* 1995;90:195.)

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CHAPTER 109

Influence of Environment and Housing on Swine Reproduction

JOSEPH M. ZULOVICH and RODERICK C. TUBBS

EFFECTS OF TEMPERATURE

The effects of temperature on reproductive performance have been examined by a number of researchers. Although the specifics may be debated, there is general agreement about the impact of temperature.[1] In general, increases in temperature above a critical level cause a decline in reproductive performance. In sows and gilts, the reduction in reproductive performance is expressed primarily as establishment of fewer pregnancies when heat stress is not severe. If heat stress is severe during breeding and early gestation, declines in reproductive performance also can be expressed as decreased embryo survival. Semen quality of boars is reduced by heat stress, but that effect may not become apparent for up to 40 days.[1] Boars exposed to a period of significant heat stress exhibit diminished semen quality for a limited period of time. The effects of heat stress on semen quality dissipate after a lag time of about 60 days after the heat stress ends.

Severe heat stress usually is characterized by sustained increases in rectal temperature. If rectal temperatures remain elevated, methods to reduce heat stress should be implemented. Such measures may include use of evaporative cooling pads, drippers and foggers, and circulating fans. To fully understand the effects of temperature, the concepts of **effective environmental temperature** (EET) and **lower critical temperature** (LCT) must be understood. EET is the temperature that a pig actually feels. EET includes the effects of air speed (drafts), floor type, building insulation, and supplemental cooling. The LCT is the EET at which pigs must increase metabolism to maintain body temperature and production.[2] If sufficient energy intake is not maintained, pigs use body reserves to increase metabolism. LCT also is defined as the EET at which cold stress begins to occur.[3] To prevent cold stress in sows, the EET should be above 15.5° C. EET can be used to evaluate the various methods for reducing heat stress.

Feed intake and subsequent metabolism in gestating sows or gilts provides energy for maintenance (i.e., basic life processes and body temperature control), pregnancy, and body condition or growth. Energy for metabolism must come from feed intake. Metabolic energy will be allocated to maintenance first, then to pregnancy maintenance and development, and last to body conditioning and growth. If sufficient energy intake is not provided, a sow or gilt will lose body condition. To stop loss of body condition, the EET must be raised or feed intake must be increased. If a number of animals are losing body condition, the EET should be raised if possible. If only a few animals are losing body condition, their energy intake should be increased. If the EET is increased for a few animals, the remaining animals in the same environment may become heat stressed. If an animal is heat stressed, it either will use additional energy to dissipate heat or will decrease energy intake. With constant energy intake, a heat-stressed sow could lose body condition because additional energy is used to dissipate body heat, through panting, for example.

EFFECTS OF HUMIDITY

Relative humidity (RH) should be maintained between 40% and 60% but must be maintained below 80%.[4] An environment that provides an RH between 40% and 60% is healthiest for sows. Most bacteria and other microorganisms thrive in an environment with high humidity. An RH between 40% and 60% minimizes the proliferation of bacteria while providing a level of humidity that is practical to obtain with typical ventilation and heating equipment. If the environment gets too dry, dust levels tend to increase. If the inside environment gets too wet (RH above 80%), moisture condensation problems typically occur when the outside weather is cold. During some periods during the year, it is nearly impossible or very impractical to maintain RH within the 40% to 60% range. During these periods, 70% RH should be the upper maximum, and good management practices, such as stringent sanitation, should be emphasized to help ensure a healthy environment for the sows.

Swine are less sensitive to humidity than to air temperature. A temperature-humidity index combines the effect of temperature, using the dry bulb temperature, and humidity, using the wet bulb temperature, into one numeric value. A temperature-humidity index for swine has been developed based on the physiologic responses of swine to various thermal environments.[5] The coefficients of the temperature-humidity index are 0.75 for dry bulb temperature and 0.25 for wet bulb temperature for adult swine. Because the coefficient for dry bulb temperature is considerably larger than the coefficient for wet

bulb temperature, adult swine are more sensitive to air temperature than to humidity. This fact is readily apparent in practice because evaporative cooling pads are extensively used in swine facilities to reduce heat stress. An evaporative cooling pad system converts energy in air based on dry bulb temperature to energy in air based on wet bulb temperature (humidity). By shifting the energy in air from a component to which large swine are highly sensitive to a component to which swine are less sensitive, the thermal environment the pig "feels" due to heat is minimized, and the environment is less stressful to the animal. If evaporative cooling pads are used, good management must be practiced so that adverse health effects due to high humidity are minimized.

EFFECTS OF LIGHT

Light frequency and intensity for swine housing typically are based on the needs of the workers and the activities they perform,[6] rather than on providing the best physiologic situation for the animals, largely because little research has been done on the influence of light, and the results of what has been done are conflicting.[7–12] The general level of illumination recommended for breeding and gestation facilities is 15 foot-candles. The level of illumination should be increased to 20 foot-candles for animal handling and inspection. For the breeding area, this would correspond to the times when hand mating activities are being conducted.

Despite conflicting research results, the effects of light on swine reproduction are beginning to be understood. Gilts attain puberty normally with a minimum of 9 hours of light per day. Boars exposed to 15 hours of light per day had better semen quality at 8 months of age than did boars exposed to natural short-day photoperiods.[2] Long photoperiods during lactation may or may not improve the wean-to-service interval (WSI) or postweaning reproductive performance. Long photoperiods during late gestation may predispose to longer WSIs.[13] A long photoperiod has been shown to increase the length of the estrous period in gilts by about 1/2 to 1 day.[14] One study has suggested that supplemental light during lactation (16 total hours of light versus normal daylength patterns in controls) synchronized the onset of postweaning estrus in sows, and a higher proportion of treated sows than control sows had WSIs of 5 days or less.[12] Other studies have shown no effect.[7–11]

EFFECTS OF AIR CONTAMINANTS

Air contaminants that typically are found in swine facilities include ammonia, dust, hydrogen sulfide, and carbon monoxide. High levels of these contaminants, such as ammonia levels above 100 parts per million (ppm), have been shown to decrease production levels and cause health problems.[1] The swine industry is rapidly moving to improve air quality in swine confinement facilities, however, to benefit the health of the animal caregivers.[15] The air quality in swine breeding and gestation facilities should be maintained within the standards for gases defined by the National Institute for Occupational Safety and Health (NIOSH). No worker health standards are currently in effect for agricultural workers. The current NIOSH standards for industrial workers are used only as a guide for agricultural workers. If gas contaminant levels are kept within these standards, air quality should not have any adverse effects on worker health. Air quality in modern breeding and gestation facilities should not adversely affect sow reproductive performance if the air quality is adequate for the workers inside the facility.

The manure management system has a major impact on the air quality inside a swine facility. Manure management systems affect the levels of ammonia, hydrogen sulfide, and methane. Ammonia levels in buildings with scraper systems can average about 25 ppm[16] or higher, and buildings with scraper systems have a difficult time maintaining air quality that is acceptable for worker health as just described. Therefore, scraper systems probably should not be considered as an acceptable method of manure removal. Deep pit buildings can have average ammonia levels that are acceptable,[17] but when pits are emptied, ammonia levels, along with hydrogen sulfide and methane levels, usually are not acceptable and can reach dangerous levels for both workers and pigs. Acceptable ammonia levels can be achieved with the use of flush systems and pit recharge systems. These systems either quickly remove manure from the building or immediately submerge manure in water. Another acceptable manure handling system is a gravity-drain, liquid manure system (hairpin gutters). This system submerges manure in liquid and then periodically removes manure from the building. When manure is either quickly removed from the building or submerged in water along with periodic removal of the liquid, air quality inside a facility usually is acceptable.

INFLUENCE OF TYPE OF HOUSING ON SOW REPRODUCTIVE PERFORMANCE

Although most data now indicate a clear advantage in reproductive performance for sows housed individually in stalls,[18,19] opinions about the best way to house sows continue to be divided. This division occurs because of disagreements about the type of system that provides the best animal welfare and because of the perceived desires of the public for some input into the management practices that produce the meat they consume.[20] It is beyond the scope of this chapter to discuss all of the social implications of sow housing. We concentrate on practices that obtain high reproductive performance while simultaneously providing for sow health and longevity.

Inside versus outside sow housing. McGlone[21] has summarized data comparing inside-housed and outside-housed sow herds in three countries (Table 109-1). These data indicate that inside sow housing has a definite advantage in reproductive performance. One problem with interpreting the data is that outside sow herds in Europe tend to be more intensively managed than those in the United States. There is general agreement, however, that reproductive performance is higher in sow herds housed inside.

Inside sow housing: individual versus group. With inside sow housing, several options are available. Sows may be housed individually, in large groups, in small

Table **109-1**

Reproductive Performance of Inside-Housed versus Outside-Housed Sows in Three Countries

	OUTSIDE			INSIDE		
Variable	**US**	**UK**	**France***	**US**	**UK**	**France**
Litters/sow/year, *n* 2.1	2.2	2.3	2.3	2.4		
Pigs weaned/litter, *n*	8.6	9.4	9.1	8.7	9.6	9.5
Pigs weaned/sow/year, *n*	19.1	20.9	21.3	19.8	21.8	22.7

*Average for all herds, not just outside herds.
Original data from Meat & Livestock Commission: *Pig yearbook,* Milton Keynes, UK, 1989 (UK data); Le Denmat MJ, et al: Outdoor pig breeding in France, *Pig News Info,* in press (French data); and Polson D: Problem-solving in swine breeding herds: methods for analyzing production problems and assessing herd-level risk factors. PhD dissertation, University of Minnesota, 1994 (US data).

Table **109-2**

Reproductive Performance of Sows in Various Housing Systems

	U.K. DATA			U.S. DATA		
Variable	**ESF**	**Groups**	**Individual**	**Individual**	**Groups**	**Pasture**
Litters/sow/year, *n*	2.31	2.25	2.27	—	—	—
Farrowing rate, %	—	—	—	83.8	78.2	78.2
Pigs born alive/litter, *n*	10.7	10.9	10.7	10.2	10.1	9.9
Pigs weaned/litter, *n*	9.4	9.5	9.5	8.98	8.42	8.01
Pigs preweaning mortality, %	12	12.6	11.3	12	16.6	19.1
Pigs weaned/sow/year, *n*	21.7	21	21.7	20.6	18.5	17.6

ESF, electronic sow feeder.
Original data from Meat & Livestock Commission: *Pig yearbook,* Milton Keynes, UK, 1994 (U.K. data); and Polson D: Problem-solving in swine breeding herds: methods for analyzing production problems and assessing herd-level risk factors. PhD dissertation, University of Minnesota, 1994 (U.S. data).

Table **109-3**

Reproductive Performance of Sows in Crates or Girth Tethers during Gestation and Lactation

Variable	**Crates**	**Girth Tethers**
Farrowing rate, %	89.6	83.3
Pigs born/litter, *n*	10.6	9.1
Pigs weaned/litter, *n*	9.4	8.1
Pigs weaned/sow/year, *n*	21.6	18.6

Original data from McGlone J: Comparison of stalls, pens, and pasture in gestation. Proceedings of the Iowa State University Professional Swine Management Certification Series, Des Moines, IA, 1995.

Table **109-4**

Reproductive Performance of Sows in Individual Stalls, Girth Tethers, and ESF Groups

Variable	**Individual Stalls**	**Girth Tethers**	**ESF Groups**
Pigs born alive/litter, *n*	10.3	10.1	10.1
Pigs weaned/litter, *n*	9.2	9.0	8.9
Pigs weaned/sow/year, *n*	20.1	19.5	19.1

ESF, electronic sow feeder.
Data from Voermans J: Recent developments in sow housing. Proceedings of the Iowa State University Professional Swine Management Certification Series, Des Moines, IA, 1995.

groups, or in groups in which feed is delivered by computer-controlled feeders (electronic sow feeders [ESFs]) that require electronic sow identification. These systems have been compared[21,22] (Tables 109-2 to 109-4). From the data, it is apparent that in the United States and the Netherlands, individual sow housing is the superior system for high reproductive performance. In the United Kingdom, ESF systems are equal to individual housing, at least when the results from stalls and tethers are combined. These results must be interpreted with some caution, because stalls and tethers are different systems and usually are associated with different levels of reproductive performance[21] (see Table 109-3).

Data from the Netherlands, collected from 1987 to 1990, support the use of individual stalls for higher reproductive performance. In this study, individual stalls, girth tethers, and group housing with electronic identification and computer-controlled feeding were compared

(see Table 109-4). Although all of the systems gave acceptable reproductive performance, individual stalls had a numerical advantage in almost all of the categories reported.

Influence of housing on the wean-to-service interval. Studies of the influence of various types of housing systems on the WSI are divided. Some studies show no influence of type of housing.[23] Others show a definite advantage for individually housed sows.[24–27] Our observations are that sows housed individually either show stronger physical signs of estrus or are more readily detected in estrus by most breeding managers.

SUMMARY

Regardless of specific housing situations, the sow's needs and her care and comfort are of primary importance. Sows usually are under some degree of stress after weaning, because of the demands of lactation and the abruptness of weaning. The endocrine system undergoes profound changes, and physical changes occur as well. In most management situations, individually housed sows require but also receive more individualized attention. Feed intake can be measured and controlled more accurately. Observation for signs of estrus and responsiveness to the presence of a boar may be facilitated by individualized housing. Like individual housing, group housing has its own unique set of potentially stressful situations. Injuries due to fighting, inadequate feed intake by some sows and excessive feed intake by others, and poor exhibition of the physical signs of estrus by sows that are low in the social order within a group all are potential problems with group housing.

Caution must be used in interpreting most of the available data on sow housing, for several reasons. First, the data in most instances is from records of field observations, not controlled studies. A controlled study would require a single farm to provide the various housing options in the same or identical buildings operated by the same or identical management. Second, statistical significance is not reported for most of the studies, only numerical differences. Third, all outdoor systems are not equal; likewise, all indoor systems are not equal, and group size is not defined in many of the reports. Finally, animal handling and human-animal interactions have a profound influence on sow reproductive performance.[28] Sows respond to their treatment. Data from farm records cannot account for differences in husbandry skills from farm to farm.

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CHAPTER 110

Reproductive Health Programs for Swine Herds

JAMES G. FLOYD, Jr and RODERICK C. TUBBS

Veterinarians often have concentrated on infectious diseases when addressing reproductive problems in swine herds. The enhanced ability to critically analyze farm records with computer databases, however, has led to the conclusion that greater than 90% of suboptimal reproductive performance is due to factors other than infectious disease, such as seasonal effects[1] or shortcomings in management.[2] Nevertheless, infectious disease cannot be ignored when it comes to optimizing reproduction. Establishing a comprehensive reproductive health program minimizes the adverse effects of disease and enhances reproductive management. Many measures to control infectious disease involve scheduled production flow, which necessarily involves optimal breeding management to provide sufficient numbers of pigs to enter that flow as a group.

Biosecurity is the term used to describe those efforts taken to protect swine herds from infectious agents—viral, bacterial, fungal, or parasitic.[3] A comprehensive biosecurity program should systematically (1) prevent disease entry, (2) maintain disease resistance, (3) monitor disease status, and (4) control disease outbreaks.

PREVENTING DISEASE ENTRY

Specific measures should be taken to prevent pathogen entry from other farms and nonpig vectors. In addition, contact between the established herd and new additions to the breeding herd must be regulated to protect both.

Location of the herd is a major determinant of the ability to deny pathogen entry.[4] Airborne spread is a major route of pathogen movement[5] (Table 110-1). Spread of pseudorabies virus has been documented in consistent directions along prevailing seasonal winds.[6] Porcine reproductive and respiratory syndrome virus (PRRSV) also may be spread by aerosols, although the data are inconclusive. Ideally, a facility should be located as far as possible from other facilities or farms, although in pig-dense areas the choices may be limited.

Nonpig vectors such as rodents, birds, insects, pet or stray dogs and cats, and humans can mechanically introduce pathogens from contact with pigs and their wastes on other farms. Constructing a barrier perimeter fence 12 to 15 m from buildings to restrict animals, vehicles, and people to one portal of entry is now standard for new construction in the swine industry.[7] Prominent signs warning visitors to stay out without prior approval and to check in before making deliveries will reinforce the importance of biosecurity to herd employees and visitors.

Establishing an apron of gravel or limestone 0.6 to 0.9 m wide and 15.2 cm deep and eliminating weeds around hog buildings, along with strategic placement of traps and baits, help to rodent-proof a building.[8] "Hardening" a building by tight construction and closing potential openings help to control rodents, flies, and birds.

Although pigs remain the primary source of introduction of new diseases, humans can be a significant mode of pathogen entry. Establishing shower-in–shower-out entry, with protective clothing provided by the farm, and disinfectant foot baths will control most of the potential for pathogen introduction by humans. Because of concerns that the human body may temporarily harbor certain swine pathogens that may not be eliminated by

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baths or disinfection, some farms have required that visitors must not have been on another hog farm at any time during the preceding 72 hours. Although such steps may decrease potential for disease entry, information regarding delay periods that are adequate or even reasonable is scarce. In addition, this requirement can significantly restrict the activities of veterinary consultants.[9]

Swine transport vehicles should be sanitized between loads. Movement of hogs should be one-way, with no reentry after the vehicle has passed the "point of no return." Disease outbreaks have been traced back to hogs that went onto a truck, only to scurry back into the finishing floor.

Incoming breeding stock are potential sources of disease entry. Although breeding stock sources recognize that a "clean" reputation is vital to continued success, a wide spectrum of health programs exist on those farms. **Minimum-disease status**, sometimes referred to as **high health**, implies a low level of pathogens without specifying those not present. Some breeding stock companies utilize these terms for the herds in their distribution pyramid.[5] Herds with **specific pathogen–free** designation are certified free of certain enumerated pathogens by virtue of cesarean derivation and immediate isolation of pigs. Those pigs are used to populate primary specific pathogen–free herds or to farrow pigs naturally, which are then used to populate secondary specific pathogen–free herds. Breeding stock from minimum-disease herds, some now preferentially located in areas not traditionally swine-dense, may be more at risk from diseases present in the herd of entry than the recipient herd is from the new animals. Quarantine and isolation of these pigs are essential, in conjunction with planned exposure to the organisms resident on the farm. Examples of recommended introduction protocols from two seedstock companies are shown in Table 110-2. In general, a 60-day quarantine period is recommended to reduce the possibility of PRRSV transmission. After 14 to 21 days, animals are serologically tested for any diseases of regulatory concern. Appropriate vaccines and exposure protocols are applied following the retesting procedure.

Artificial insemination (AI) and embryo transfer technology may offer opportunities for reducing the need to introduce new animals into a breeding herd. At present, AI is a more practical technology than embryo transfer for routine use. In most situations, use of AI can improve health status.

Buying and introducing fewer boars because AI is being used constitute one reason AI can decrease disease risk. A 600-sow farm with 30 boars probably will buy 10 new boars per year. A 600-sow farm using AI and maintaining 6 boars is likely to purchase only 2 new boars per year. Fewer introductions of new animals reduce the risk of introducing disease with the new animals.

An off-site boar stud facility may offer an opportunity for improved biosecurity in some systems of production. An off-site facility requires at least the same level of biosecurity as that practiced in the breeding herd. To gain the full benefit of improved biosecurity with a boar stud, separation between collection technicians and processing technicians should be maintained in the AI laboratory. A pass-through window or a processing area separated from the collection area is needed. Collection technicians should never enter the laboratory area.

Buying semen rather than boars may further reduce the risk of introducing disease; however, the source herd

Table **110-1**

Airborne Pathogen Spread

Pathogen/Disease	Maximum Distance, (km)
Transmissible gastroenteritis virus	0.8
Atrophic rhinitis	0.8
Pleuropneumonia	0.8
Mycoplasma hyopneumoniae	3.2
Porcine reproductive and respiratory syndrome virus	3.2
Swine influenza virus	4.8–6.4
Pseudorabies virus	40.2
Foot-and-mouth disease virus	40.2

Data from Moore C: Biosecurity and minimal disease herds. *Vet Clin North Am Food Anim Pract* 1992;8:461.

Table **110-2**

Breeding Stock Company Protocols for Introducing Animals

Program Feature	Company 1	Company 2
Duration of isolation	30–42 days	28 days, 28 days acclimatization
Distance of isolation facility from herd	Minimum 91 m; 0.8–1.6 km desirable	1.6 km minimum; 3.2 km desirable
Commingling	Adjacent pen contact with animals from herd (including culled sows OK) after 1 week	Nose-to-nose contact with culled boars and sows
Feedback	Fresh manure from breeding herd three times a week for 2 weeks	Manure, placentas, and dead fetuses continuously for 3–4 weeks (in consultation with herd veterinarian's DVM)
Vaccination	Same vaccines used in existing breeding herd	Leptospirosis, parvovirus, erysipelas (others in consultation with herd veterinarian's DVM)

should be checked in the same way as that used for screening live animals for purchase. Several species of bacteria are commonly found in boar semen. Many of them are commensal inhabitants of the preputial area and are not specific pathogens for the reproductive system but nevertheless may disrupt individual pregnancies. A major problem with many of the contaminating bacteria is that they reduce the shelf-life of semen. Some bacteria may have a spermicidal effect as well. Because the temperature at which swine semen is stored (15–20° C) does not inhibit bacterial growth, antibiotics are routinely added to semen extenders.

Many viruses have been shown to be present in boar semen. Only three, African swine fever virus, porcine parvovirus, and PRRSV, have been proved to be transmitted to sows through semen. The same precautions must be taken with the source of semen as are taken with live animals, to minimize the risk of bringing these viruses into the herd with semen. Because viruses and their by-products usually are not spermicidal, and antiviral agents are much more expensive than antibiotics, antiviral agents are not commonly added to semen extenders.

In addition to the potential for reducing the risk of disease from incoming animals, AI can reduce the spread of disease organisms within a breeding herd. For example, on a 600-sow farm, 30 boars are more likely to transmit disease organisms within a breeding herd than are 6 boars that never actually come into physical contact with the sows. At least some anecdotal evidence suggests that correct use of AI can limit vaginal discharge problems within a sow herd.

MAINTAINING DISEASE RESISTANCE

However free a herd may initially be from infectious pathogens, this status tends to erode over time. The speed and degree of erosion depend on the biosecurity measures employed to protect the herd from entry of outside pathogens, and on measures taken to decrease pathogen transmission and multiplication within the herd.

Within a herd, infectious agents such as coccidia, ascarids, and clostridia can be present in the environment long after pigs have been removed.[5] The pig itself, however, is the source of most pathogens, with disease transmission within a herd occurring by direct pig-to-pig contact.[5] The cycle of transmission for both environmental and pig-to-pig sources can be broken through production flow technologies that minimize contact of susceptible pigs with pathogens present in other pigs or the environment.

Production flow should be managed so that groups of pigs start out life in a closely matched age group and stay together in that group until finished or introduced into the breeding herd as replacements. **All-in–all-out** production systems move pigs through facilities as a group so that the facilities can be totally emptied on a schedule, allowing adequate time for clean-up between groups. This reduces the chance of infection of the next group of pigs from an unsanitary environment. Perhaps even more important, all-in–all-out production keeps pigs of similar age and immune status together, without exposing them to older pigs that may be shedding organisms to which younger pigs may not have developed immunity.

Table **110-3**

Maximum Weaning Age to Eliminate Pathogens

Organism	Maximum Weaning Age, (d)
Mycoplasma hyopneumoniae	<10
Pasteurella multocida	<10
Salmonella choleraesuis	<12
Haemophilus parasuis	<14
Actinobacillus pleuropneumoniae	<21
Pseudorabies virus	<21
Transmissible gastroenteritis virus	<21

Segregated early weaning is another production flow technology that refines the all-in–all-out system. Because the breeding herd serves as the resident source of most infectious pathogens on a farm, separating pigs from the breeding herd as soon as possible before pathogen transmission takes place is a logical step (Table 110-3). Weaning before 21 days of age accomplishes this goal at a time when the pig's colostrum-derived immunity is still sufficiently high to provide some protection. Traditional weaning at 3 to 4 weeks of age coincides with the time at which colostral immunity has waned, when the pig's immune system is just beginning to respond to the organisms it encounters.

Coupling segregated early weaning with **two- or three-site isolated production** adds a further biosecurity element to the all-in–all-out system. Two-site production includes weaning pigs at 10 to 21 days old and moving them to a nursery on the same site as the finishing unit, usually located at least 1.7 km away from the farrowing site. With three-site production, the early-weaned pigs are moved to an isolated nursery and then at approximately 18 to 27 kg are moved to a finishing facility at a third site.

Enhanced production flow technologies allow the manager to optimize each unit's environment for the age of the pig it contains. Temperature and ventilation requirements can be maintained in a more cost-effective manner if the pigs are within a narrow range of ages, while an environment is created that is more conducive to pig comfort and reduced pathogen transmission. Accurate production scheduling allows for accurate scheduling of farrowing, which facilitates induced and attended farrowings to lower intrapartum and peripartum piglet mortality.

Although vaccine efficacy data may not accurately reflect the degree of protection against reproductive or other diseases in field settings,[10] routine vaccination of breeding swine for common diseases is still recommended. Prebreeding vaccination of sows and gilts and twice-yearly vaccination of boars against leptospirosis, porcine parvovirus infection, and erysipelas are routinely recommended by most veterinarians. Prefarrowing vaccinations against *Escherichia coli* and atrophic rhinitis also

are widely used. In some segregated early weaning protocols, especially in the initial **medicated early weaning** and **modified medicated early weaning** programs, extensive prefarrowing immunization protocols have been recommended to maximize colostral antibodies for pigs to undergo early weaning. Actual vaccination protocols should be based on disease monitoring programs established by a herd veterinarian who is intimately associated with the production flow and management of the herd. The specific protocols should be reviewed by the veterinarian and the on-farm manager at least semiannually.

MONITORING DISEASE STATUS

Programmed herd visits in conjunction with careful monitoring of production data through computer databases are the basis of veterinary consultation in modern swine herds. The expanded use of databases has allowed for better definition of "normal" production figures and for establishment of intervention levels when those figures lie outside the normal range[2] (Table 110-4).

Serologic monitoring may be a useful adjunct to regular veterinary consultation; however, immunodiagnostic profiles must be interpreted carefully because the degree of antibody response may vary tremendously depending on the laboratory, the sensitivity and specificity of the particular test, the sample size, and the time of collection with respect to incidence of clinical or subclinical disease.[5,11,12] It is important to recognize that a rising antibody titer indicates exposure to a particular organism. Exposure, however, does not necessarily equal causation.

Serum banked from blood collections for regulatory purposes may be useful for retrospective diagnosis of disease emergence. With specific reference to reproductive problems, however, relying on serologic testing alone may lead to the conclusion that infectious disease is at fault, when other factors are more likely.

Table **110-4**

Target and Intervention Levels for Commonly Used Reproductive Parameters

Parameter	Target	Intervention Level
Farrowing rate, %	87	80
Liveborn, *n*	10.5	10
Stillborn, %	5	8
Mummies, %	1	3
Birth weight, kg	1.6	1.4
Weaned, *n*	9.3	8.5
Preweaning mortality, %	10	15
21-day adjusted wean weight (litter), kg	59.1	50
Litters/sow/year, *n*	2.5	2.2
Pigs/sow/year, *n*	23	20
Pigs/crate/year, *n*	130	110
Cull rate, %	35–40	<30; >50
Nonproductive sow days	40	60

Necropsy of pigs that die is desirable if it can be practically accomplished. It may not be feasible for every dead pig to be examined; however, if mortality exceeds the established intervention level, it should become a priority. Necropsy can be facilitated on some farms by training competent employees to collect applicable tissues and forward them to the prearranged diagnostic laboratory. Similarly, some consultants have instructed workers to freeze dead neonatal pigs for gross necropsy during the regular visit. In this fashion, stillborn pigs can be distinguished from those with inflated lungs, and gastrointestinal disease sometimes can be detected as a cause of death.

Slaughter checks are useful for veterinary consultants to track diseases of the respiratory and gastrointestinal systems, if sample size is sufficient for the size of the herd and disease prevalence.[13] It must be kept in mind that gross evaluation of slaughterhouse specimens of finished hogs provides information on disease that has occurred during the last 2 months of production. Indeed, some recent studies have failed to demonstrate a significant relationship between snout and lung lesions at slaughter and measures of growth performance.[14,15]

Examination of reproductive tracts of breeding age animals at slaughter may aid in the diagnosis of noninfectious causes of reproductive failure and infectious conditions of the urogenital tract. Coupled with knowledge of normal ovarian, uterine, and fetal morphology and physiology, examination findings may help the consultant to formulate appropriate intervention strategies.[16]

CONTROLLING DISEASE OUTBREAKS

Effective treatment begins with accurate diagnosis. Acute "storms" of infectious disease may dramatically affect reproductive parameters, such as occurred with the previously unrecognized PRRSV in the late 1980s. Reproductive signs of acute PRRS include premature farrowings, increased stillbirths, mummification of fetuses, and birth of weak piglets.[17] Reproductive problems, however, usually do not occur as storms but more often are manifested as decreasing production levels.

When seeking the cause of reproductive performance shortfalls, swine veterinarians must evaluate (1) people, (2) environment, (3) nutrition, (4) genetics, and (5) health.[2] A correct and timely diagnosis is more likely with systematic investigation of these potential factors.[18]

Critical analysis of farm records in light of management changes over time may be the most effective diagnostic tool available. For example, making personnel changes in the breeding gestation unit may be reflected by a decreased weaning-to-first-service interval, although this may not be seen for some time after the new person takes over.[2]

Although resort to medications often is the first response when reproductive problems are recognized, such agents have seldom been demonstrated to be efficacious. This probably is reflected in the scarcity of label approval of drugs for swine reproductive diseases. Oxytetracycline and chlortetracycline as feed additives have label uses for reducing the abortion rate in the presence

of leptospirosis, and injectable erythromycin and oxytetracycline are labeled for swine leptospirosis.[19]

Vaccinations at critical periods may increase resistance to disease and reduce its effects. Such periods can be identified serologically in some instances. Vaccination is more often utilized with diseases caused by agents such as *Actinobacillus pleuropneumoniae* than with reproductive disease.

Depopulation-repopulation may be indicated when disease has become endemic in a herd. The cost-benefit ratio of such a strategy should first be carefully studied because of the concurrent disruption in cash flow and significant expenses incurred. In addition, this extreme strategy may not solve "people" problems if the same personnel are present after depopulation. Furthermore, certain reproductive problems such as vaginitis and cervicitis, viral embryonic loss (e.g., from parvovirus or enterovirus infections), effects of parity distribution, and leptospirosis may occur despite depopulation-repopulation.[20]

Creative strategies such as **partial depopulation** of the nursery facility for a limited period have been successfully used to eliminate nursery deaths associated with chronic PRRS.[21] This strategy was based on the recognition that pigs did not seroconvert until 8 weeks of age, and these pigs were infecting younger pigs when they were introduced to the nursery. Such partial depopulation allowed the preservation of valuable breeding stock and minimized the loss of cash flow.

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CHAPTER 111

Using Statistical Process Control to Investigate Reproductive Failure in Swine

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You are called by a producer who is alarmed that the farrowing crates are half empty this month. What should he do? Another producer calls to report yet another abortion. Should he vaccinate? A third producer calls: She has noticed an increase in the number of returns to service. What should she do?

The diagnostician investigating a case of reproductive failure usually is presented with a primary clinical sign. There are relatively few manifestations of reproductive failure but many potential causes of each. Reproductive failure may be clinically manifested by one or more of the following signs:

- Failure to show estrus
- Return to estrus after being bred
- Abortion or premature parturition
- Failure to farrow despite having conceived
- Abnormal litter characteristics at birth as evidenced by low number of liveborn piglets or an elevated number of stillborn piglets, mummified piglets, or weakborn piglets

For example, one might be presented with a low farrowing rate; that is, a lower than expected percentage of sows that were apparently bred are farrowing. To investigate this case, the diagnostician must first know what the expected farrowing rate is for this herd. Assuming a "real" decrease has occurred, the diagnostician then must determine if the sows were actually bred and whether they conceived, and, assuming they conceived, why they did not farrow (Fig. 111-1). A relatively simple-appearing clinical situation quickly becomes complex. The case is further complicated if the producer lacks detailed reproductive records (arguably, without good records, the case is simple—it is unsolvable).

In investigating a case of reproductive failure, it may help to remember that the breeding herd exists to produce weaned pigs. The number of weaned pigs per unit of time is referred to as the herd's **output** and is determined using the following formula:

number of females bred × farrowing rate × average liveborn × survival rate = output

For example:

30 bred per week × 80% farrowing rate × 10 liveborn × 90% survival = 216 pigs weaned

The gilt pool and breeding, gestation, and farrowing facilities will have a certain capacity in terms of pigs weaned per unit of time, and the actual number achieved divided by the potential is referred to as the **average utilization rate.** This commonly is expressed as a percentage. As might be expected, unused capacity costs money (i.e., the opportunity cost attributed to pigs that could have been produced but were not), and the usual intention is therefore to maximize the utilization rate. The first step in maximizing utilization is to breed the appropriate number of females per unit of time. This having been accomplished, any real decrease in utilization rate may be caused by reproductive failure. The diagnostician's job is to identify the source of the problem and implement solutions.

Returning to the farrowing rate example, suppose a herd's farrowing rate has decreased from 85% to 70%. The diagnostician must now determine (1) whether the clinical manifestation represents a "real" change or random variation and (2) if the problem is "real," what the cause of that problem is. The purpose of this chapter is to guide the diagnostician through these decisions.

APPROPRIATE DECISION MAKING: WHEN IS A SYSTEM OUT OF CONTROL?

Reproductive failure may present itself as an epidemic within a herd (sudden onset—an "outbreak") or as a chronic, lower-than-expected level of performance. Diagnostically, the method of investigation and the likelihood of identifying the cause differ markedly between these two presentations.

Epidemic Reproductive Failure

In our experience, epidemics of reproductive failure are more likely to be caused by a single infectious agent such

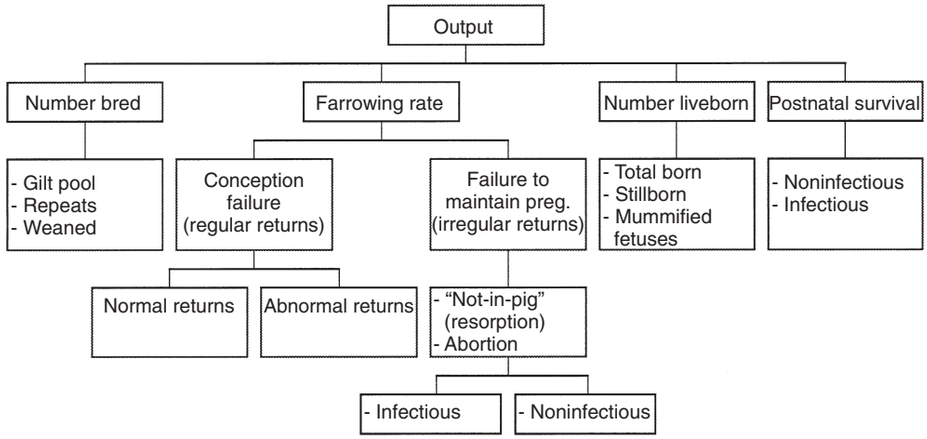

Fig. 111-1 Summary flow chart for investigating reproductive failure.

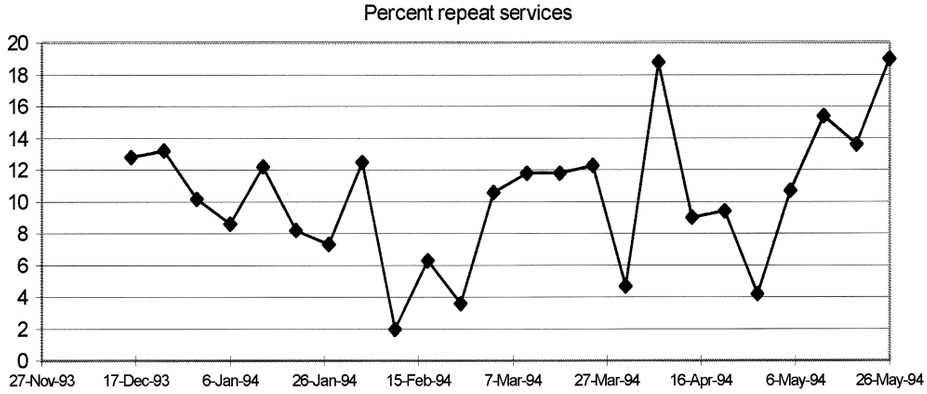

Fig. 111-2 Run chart of percent of sows returning to service in a 1400-sow herd.

as PRRSV or porcine parvovirus. A sudden, but less dramatic, decline in reproductive performance may be associated with epidemics of infection due to pseudorabies virus, transmissible gastroenteritis virus, or swine influenza virus. These latter cases of mild but significant reproductive failure also may be caused by a variety of bacteria, including *Leptospira* spp. Diagnostically, submission of appropriate samples from a herd epidemic is likely to be very revealing if an infectious agent is involved.

Noninfectious causes of reproductive failure also can present as epidemics. This might include such factors as the use of infertile boars, a change in breeding techniques, poor feed quality, seasonal infertility, and others.

How does the diagnostician know when a herd is experiencing an epidemic of reproductive failure? An **epidemic** may be defined as the number of failures being "one more than expected." Unfortunately, managers and consultants usually are not sure of what is expected. Although an intuitive impression may be correct, veterinarians need to be cautious as they evaluate herd performance and make recommendations. Effective decision making entails (1) knowing when a real change has taken place and not random variation, (2) evaluating the cost-benefit ratio of options, and (3) deciding when the problem should be reevaluated.

Two types of errors are possible in making such decisions. A problem might be diagnosed and a treatment or prevention instigated when the apparent problem was really random variation (type I error). It is this type of error that is responsible for many needless vaccination or medication programs. Our bias is to err on the conservative side and intervene when an intervention is not called for. A second type of error (type II) occurs when a situation is examined and intervention is not elected when, in reality, corrective measures were indicated. That is, the situation was "out of control" and action really *was* necessary. The first step is to chart the data. Columns of data are extremely unrevealing, and graphing techniques have been developed that help present and interpret the trends.

A **run chart** is a type of line graph on which time is on the *x*-axis and the variable of interest is on the *y*-axis (Fig. 111-2). Run charts are very easy to compose and allow quick interpretation of trends. However, it is difficult to differentiate between random fluctuations and early, mild trends in the data. Therefore, another type of chart called a control chart was developed.

The **control chart**, like the run chart, plots the actual values over time but also considers the recent variation while estimating control limits (Fig. 111-3). Control limits are calculated by adding and subtracting three standard deviations to the average value. A critical step in using control charts is to start with a process that is "in control" to determine the expected standard deviation.

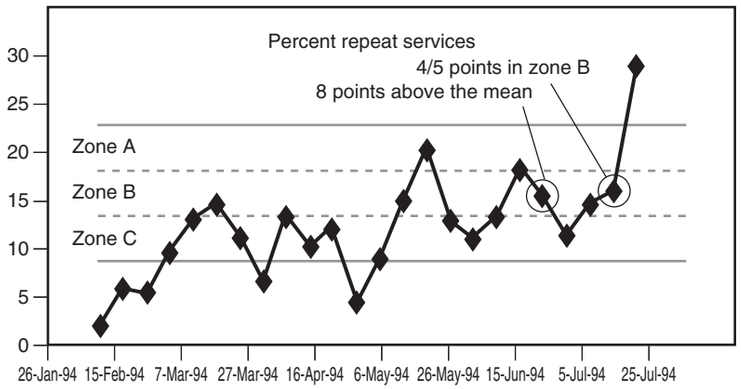

Fig. 111-3 Control chart of percent of sows returning to service in a 1400-sow herd.

Using these expected standard deviations, if the number of returns to service per unit of time is beyond the upper or lower control limit, one can be extremely confident that the process is "out of control." At this point, the consultant must intervene and initiate a diagnostic investigation.

Control charts can be further modified to create an "early warning system." That is, the area between the average and the control limit can be subdivided into three zones based on standard deviations (see Fig. 111-3). Zones C, B, and A correspond to the average ±1, ±2, and ±3 standard deviations, respectively. If the measurement of interest is normally distributed around the average, then data points should appear approximately normally distributed within the limits. Also, there should be no evidence of trends or recurring cycles. Approximately 66% of the measurements should be in zone C. Approximately 95% of the measurements should be in zone B or C, and 99% of them should be in zone A, B, or C. Therefore, only 1 out of 100 times will a measurement be expected to be outside the control limits by chance alone. In other words, if a measurement occurs outside the control limits, one can be very confident that something has disrupted the process. The zones also can be used in conjunction with statistical probability to establish some guidelines that will detect a process out of control sooner than waiting for the control limit to be surpassed.[1] The detection criteria are as follows:

- Presence of 2 out of the last 3 points in zone A
- Presence of 4 out of the last 5 points in zone B
- Presence of 8 successive points outside zone C
- Linear trend: 6 successive points with continuous increase or decrease
- Oscillation: 14 successive points oscillating up and down
- Presence of at least 8 successive points above or below the centerline, indicating that the mean has changed
- Presence of 15 successive points in C zone, indicating that the process variability has decreased

With the multitude of measurements being taken within farms that veterinarians are serving, it is impossible to monitor them all manually. Relatively simple computer programs, however, can scan all measurements for all farms on a routine basis to quickly detect any process that is getting out of control.

Endemic Reproductive Failure

In our experience, chronic suboptimal reproductive performance is considerably more difficult to investigate than epidemic reproductive failure. A myriad of possible factors can affect performance, including genetics, management, environment, nutrition, feed quality, infectious agents, and season. The goal is to optimize output from the reproductive herd. Accomplishing this entails three steps:

- Determine the reproductive capacity of the herd.
- Determine what components of reproductive performance are suboptimal.
- Identify what might be done to improve them.

As an example, a herd has a nursery/grow-finish capacity for 250 pigs weaned per week and has averaged 200 per week over the last year. The standard deviation for pigs weaned per week at this herd is 80. Is this herd achieving its potential? If it is not, where does the diagnostician start? Recall that pigs weaned per week is a reflection of number of sows bred, farrowing rate, number of liveborn pigs, and survival rate of pigs (see Fig. 111-1). In this case, the diagnostician must set a realistic goal or target for each of these components. Second, and equally important, an attempt should be made to reduce variation in each component between time periods. Two herds might both average 20 pigs per sow per year, but one might wean 250 per week ± 20 and the other might wean 250 ± 80. The former herd has considerably less disruption in planning pig flow, labor requirements, feed requirements, and cash flow.

Achieving targets and reducing variation require the diagnostician to be comfortable with data analysis and interpretation. In swine herds, production information can be collected with well-designed systems such as PigCHAMP.* Unfortunately, most record systems either bedazzle or intimidate most managers. Those who are

*Developed by the University of Minnesota, St. Paul, MN.

afraid shun them, whereas others often become prisoners of their fascination. The goal of herd advisors is to first understand how managers make decisions. In other industries, most managers do not rely on computer-based information to make decisions.[2] These managers get two thirds of their information from face-to-face or telephone conversations and acquire the remaining third from documents, most of which are from outside the organization. Davenport has summarized the facts of life regarding information:

- Most of the information people really care about is not on computers.
- Managers prefer to get information from people, rather than from computers; people add value to raw information by interpreting it and adding context.
- The more complex and detailed an information management system is, the less likely it is to change anyone's behavior.
- An element of flexibility and disorder in the manner of presentation is desirable.
- If information is power and money, people will not share it easily.
- The willingness of people to use a specified informational format is directly proportional to how much they have participated in defining it or trust others who did.
- To make the most of electronic communications, one must first learn to communicate face-to-face.
- Raw data are not information; and accumulating data is not the same as interpreting and putting these in a usable form.
- There is no such thing as information overload; if information is really useful, our appetite for it is insatiable.

Returning to the example herd, suppose it has a nursery capacity for 250 weaned pigs per week. This can be achieved if (1) 30 females are bred, (2) an 85% farrowing rate is achieved, (3) an average of 10.5 pigs are born alive, and (4) 93% survive to weaning. Furthermore, it is desirable to achieve this level of output as consistently as possible (with minimal variation). The major source of variation in pigs weaned per week is the number of sows served per week. Breeding the desired number of females is a managerial decision that requires ensuring that there is an adequate number of sows to be weaned and of gilts in the pool to supplement the expected number of returns to service.

Achieving an average farrowing rate of 85% is dependent on the conception rate and the pregnancy retention rate. Each of these needs to be approximately 92% to achieve a farrowing rate of at least 85% ($92\% \times 92\% = 85\%$). A more achievable goal is to strive for at least a 90% conception rate and at least a 95% pregnancy retention rate. The most practical method to measure conception rate is to monitor the number or percent of females that return to service.

A number of factors influence litter size.[3] Average total-born litter size is influenced in large part by maternal genetics and parity distribution of the herd. During gestation, certain feed-borne mycotoxins and several infectious agents such as porcine parvovirus, pseudorabies virus, porcine reproductive and respiratory syndrome virus, and *Leptospira* spp. can decrease the average number of liveborn pigs. The parturition process plays an important role in decreasing the number of stillborn pigs, thereby increasing the proportion born live. Finally, the farrowing environment can influence the survivability of liveborn pigs.

After capacity of the herd and the facilities have been assessed and targets have been set for the number of services per time unit, the diagnostician must determine if the herd is experiencing endemic reproductive failure, and if so, at what point in the management process? As discussed earlier, control charts can be used to identify as early as possible when a process is out of control. The other application for control charts is to help gradually improve a process over time. This is done by reducing the variation in the system. By reducing variation over time, the control limits are narrowed and the process is continually improved.

To understand how the variation is reduced, the investigator must (1) identify reasons why the process is either above or below the target, (2) calculate the frequency of reasons, and (3) focus the investigation on the most frequent reasons for failure. This investigative process is referred to as **Pareto analysis.** The Pareto principle, also commonly called the **80/20 rule**, is that most of the instances of a process not being on target can be attributed to relatively few causes: the "vital few." That is, investigation and resolution of a vital few causes are needed to address the greater part of the problem. This strategy is in contrast with the dilution of impact that occurs in attempting to solve the "trivial many." In addressing the vital few, management concentrates on preventing these causes from recurring. For example, in some herds, at least 80% of the stillborn piglets are delivered by less than 20% of the sows. Identifying these "vital few" sows that cause a majority of the stillbirths will allow appropriate action to be taken.

Finally, once the herd is performing as desired, the investigator must ask whether the herd is as "in control" as it can be or whether there is still room for improvement. One of the caveats of control charts is that a system may appear to be in control when in fact there is much room for improvement. Benchmarking provides this answer by determining what reproductive performance is being achieved in other herds both for absolute values and for variation (Tables 111-1 to 111-3).

EXAMPLE HERD

In early August, the owner of a 1400-sow herd becomes suspicious that a reproductive problem may exist. He is particularly concerned with an apparent increase in sows returning to service. Returns to service can be identified as "normal" or "abnormal," the distinction being whether sows return to estrus at normal intervals (18–23 or 36–46 days after initial service). Normal returns to service are thought to reflect failure to conceive, whereas returns to estrus at abnormal intervals are more likely to reflect a problem independent of the breeding process, possibly an infectious disease. In view of the apparent

Table **111-1**

Descriptive Statistics of Reproduction Performance in 27 Herds with 300 to 325 Sows*

Statistic	Average	Standard Deviation
Services/week, *n*	16.4	5.5
Returns to service, %	1.8	1.4
Farrow rate, %	82.5	11.5
Adjusted farrow rate, %	85.4	10.8
Pigs liveborn/litter, *n*	10.2	2.8
Preweaning mortality, %	12.1	7.6
Total pigs weaned/week, *n*	134.5	47.8
Pigs weaned/sow/year, *n*	19.5	1.6
Pigs weaned/mated sow/year, *n*	20.0	2.0

*Selected from the PigCHAMP 1993 database, University of Minnesota, St. Paul, MN.

Table **111-2**

Descriptive Statistics of Reproductive Performance in 11 Herds with 600 to 650 Sows*

Statistic	Average	Standard Deviation
Services/week, *n*	32.2	5.3
Returns to service, %	3.6	2.4
Farrow rate, %	81.0	7.6
Adjusted farrow rate, %	84.8	7.6
Pigs liveborn/litter, *n*	10.1	2.6
Preweaning mortality, %	12.2	5.6
Total pigs weaned/week, *n*	235.0	43.8
Pigs weaned/sow/year, *n*	19.4	1.5
Pigs weaned/mated sow/year, *n*	20.4	2.2

*Selected from the PigCHAMP 1993 database, University of Minnesota, St. Paul, MN.

Table **111-3**

Descriptive Statistics of Reproductive Performance in 8 Herds with 1200 to 1300 Sows*

Statistic	Average	Standard Deviation
Services/week, *n*	64.0	8.2
Returns to service, %	6.0	2.8
Farrow rate, %	84.1	6.4
Adjusted farrow rate, %	86.8	6.0
Pigs liveborn/litter, *n*	10.2	2.7
Preweaning mortality, %	13.6	4.6
Total pigs weaned/week, *n*	484.0	65.7
Pigs weaned/sow/year, *n*	20.0	1.8
Pigs weaned/mated sow/year, *n*	20.8	2.1

*Selected from the PigCHAMP 1993 database, University of Minnesota, St. Paul, MN.

increase in returns to estrus in this herd, should a diagnostic investigation be initiated?

Using a control chart, it can be seen that on July 25 the herd was "out of control" for repeat services (see Fig. 111-3). Intervention is indicated; by failure to initiate intervention, a type II error is committed.

Aborted fetuses from two sows and stillborn piglets and large mummified fetuses from two other sows were submitted for complete diagnostic examination. No causative agent was identified. Convalescent sera were collected from 15 representative gilts and sows that had returned to estrus or aborted. All samples were seropositive for porcine parvovirus and seronegative for pseudorabies virus and *Leptospira* spp., and 9 of 15 were negative for PRRSV. Further serologic tests from acute and convalescent sows would lend evidence for any etiologic role of PRRSV.

The proportion of normal to abnormal returns to estrus was analyzed for significant change. When the herd was "in control" (April–May), the ratio of regular to irregular returns to estrus was 6.0. During the epidemic (July–August), the ratio was not markedly different at 5.7 (Fig. 111-4).

The herd owner reported that on June 1 a new employee started work in the breeding area. In this herd, AI was used almost exclusively, and the new employee, who claimed to be very competent in this technique, was put in charge of breeding. Simultaneous with this change, the source of replacement gilts was changed. The new source was thought to be of considerably higher health status than the usual source.

This diagnostic investigation indicated that the repeat breeding pattern reflects primarily failure to conceive and probably is of noninfectious origin. In all likelihood, this failure was due to incorrect semen handling, heat detection, or insemination technique, alone or in combination.

The control charts were extremely useful for easily detecting the point in time when the percent of repeat breeding went "out of control." On examining the control chart, it can be seen that on July 1, the last eight points were above the centerline, indicating that the historical average percent repeat services had increased. This is the first significant warning that the breeding process has gone awry. Two weeks later, four of the last five points

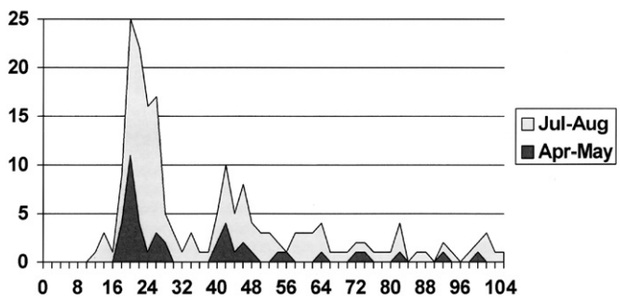

Fig. 111-4 Frequency of sows returning to service at various days after initial service from April to May versus July to August in a 1400-sow herd.

are seen to be in zone B or beyond, another significant warning. A diagnostic investigation should have been initiated 3 to 4 weeks before the producer became aware of the increase.

Control charts identify a process when it becomes "out of control" and are helpful to veterinary diagnosticians in deciding when to intervene. Even more important, however, control charts are powerful tools for reducing variation that is endemic in a process. In the example herd, it was assumed that the repeat service data were "in control" during March and April. It may be possible to reduce variation in repeat services over time from the current standard deviation of ±4.9. In so doing, a process improvement has been made that will reduce variation in output per unit time and also improve the early warning system for when the herd parameters are "out of control." By monitoring variation, consultants and managers can gradually become aware of its impact on the system and begin to realize the financial benefits of reducing it.

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CHAPTER 112

Assisted Reproductive Technologies in Swine

†ANTONIO GARCIA

Manipulation of animal reproduction probably is as old as domestication itself. Since humans started to keep animals in captivity, they have exerted a profound influence on the natural behavior of domesticated species, including reproduction. Assisted reproductive technologies will allow continued genetic advancement in farm livestock. Artificial insemination (AI), used by the Arabs in ancient times for the dissemination of male gametes and first described for publication by Lazzaro Spallanzani in 1784, probably was the first technique of modern "assisted reproduction." The first embryo transfer work, in rabbits, by Walter Heape in 1891 and the subsequent successful embryo transfers in many other species over the next century turned out to be the first step in increasing the female role in reproduction. Other assisted reproductive technologies have been developed and applied to swine under research settings. The in vitro fertilization (IVF) of oocytes and micromanipulation of domestic animal embryos, first started in the early 1980s, nowadays are accepted as commonplace and as vital components of several procedures that are rapidly transforming the embryo transfer industry. In addition to these technologies, embryo cryopreservation, intracytoplasmatic sperm injection (ICSI), oocyte transfer, and nuclear transfer are dramatically altering the very manner in which we view our own reproductive capacity. It is important to recognize, however, that assisted reproductive technologies in general do not directly apply from one species to another, as has been well illustrated by the work with pigs.

The first successful pig embryo transfer was reported in 1951 (Table 112-1), the same year as for cattle. Of all of the large domestic animal species, however, the pig is the animal for which implementation of these reproductive technologies on a production basis has been the slowest. Several unique features of the physiology of reproduction in swine, as well as technical limitations, have slowed the widespread use of embryo transfer in pigs relative to its application in the cattle industry. Part of this hesitancy can be attributed to the greater delicacy and sensitivity of swine gametes and embryos to manipulation and in part to the perception that little is to be gained owing to the high reproductive efficiency that this species naturally possesses. An additional impediment to

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Table **112-1**

Key Milestones in Development of Assisted Reproductive Technologies in Swine

Year	Event	Study
1951	First successful surgical embryo transfer	Kvasnickii[2]
1970	Successful intercontinental transport of embryos	Wrathall et al.[3]
1976	Embryo transfer used to establish SPF herd	Curnock et al.[4]
1983	Embryo transfer used to repopulate swineherds in connection with eradication of pseudorabies virus	James et al.[5]
1985	Production of transgenic piglets	Hammer et al.[6]
1985	Piglets produced from split embryos	Rorie et al.[7]
1986	Piglets produced by in vitro fertilization	Cheng et al.[8]
1989	First cloned piglet born after embryo cell nuclear transfer	Prather et al.[9]
1989	Piglets produced after transfer of frozen-thawed (–35° C) embryos	Hayashi et al.[10]
1990	Piglets produced after transfer of frozen-thawed (–196° C) embryos	Oguri[11]
1991	USDA produced first swine offspring with semen sorted for sex, after surgical transfer	Johnson[12]
1993	Production of piglets by nonsurgical embryo transfer	Reichenbach et al.[13]
1994	First piglets produced after oocyte transfer (GIFT)	
1997	First piglets of preselected sex produced after transfer of embryos fertilized with X- and Y-sorted sperm	Rath et al.[14]
1998	First piglets born after embryo vitrification—USDA	Dobrinsky et al.[15]
2000	First piglet born after fertilization of an oocyte by intracytoplasmic sperm injection (ICSI)	Kolbe and Holtz[16]
2000	First cloned piglets born after somatic cell nuclear transfer	Polejaeva et al.[17]

GIFT, gamete intrafallopian tube transfer, SPF, specific pathogen–free; USDA, U.S. Department of Agriculture.

the greater use of reproductive technologies in swine is the highly variable response to synchronization and superovulation of donor animals; furthermore, collection and transfer of porcine embryo thus far have been accomplished only by surgery. The size of the donor and unique anatomic characteristics of the reproductive tract appear to make nonsurgical recovery an unlikely prospect. Although surgical embryo transfer has been successfully used, methods for the nonsurgical transfer of embryos into recipients are still being developed. The swine embryo transfer activity has just started to grow around the world, with an estimated 7000 embryos transferred either experimentally or commercially in 2000.[1] Until now, however, the swine industry has never successfully implemented a commercial viable embryo transfer process as an alternative method of improving hog genetics. Despite the limitations, embryo transfer and related reproductive technologies hold promise as a means to accelerate genetic improvement and to take advantage of superior breeding sows, to break the link between health status of customer and source herds, to eradicate disease from a swine herd, and to facilitate inexpensive transport of genetic material around the world. This chapter presents an overview of recent work in this field, to collate existing knowledge and to establish guidelines for future work.

ARTIFICIAL INSEMINATION STRATEGIES

Reduction in Sperm Number per Dose and Intrauterine Insemination

A conventional insemination technique in swine requires 2×10^9 to 3×10^9 sperm cells per dose and a volume of insemination per dose between 80 and 100 ml. Currently, however, interest has increased in the novel possibility of introducing a lower number of sperm cells per dose or lower volumes of insemination per dose directly into the uterus. In contrast with the traditional intracervical insemination method, instruments and techniques are currently being developed to allow the semen to be deposited beyond the sow's cervix and into the uterine horns. This deep uterine insemination technology will have obvious economic repercussions in the overall productivity of boar stud facilities, and the cost per dose of semen may be reduced accordingly. Concepts for this technology began evolving in the early 1990s in an effort to help reduce the effects of summer infertility in hot climates by improving conception rates with use of poorer-quality semen. More recently, the main thrust for further refinement of this technique has been to advance the application of sperm sorted for sex and to develop a method for performing nonsurgical embryo transfer. Several manufacturers, in connection with research institutions, have created a growing array of catheter devices that are can be passed through the cervix of the sow to inseminate beyond the cervix. To date, however, research data are not sufficient to confidently establish the necessary cell numbers, semen volume, and semen placement location. Research reports demonstrate that the number of semen per dose could be reduced significantly without a corresponding decrease in either fertility or litter size. Recent results using a fiberoptic endoscope technique for deep uterine horn insemination in sows demonstrated that using only 50 to 200 million sperm cells per dose was now possible.[18] This number represents only a fraction of the 2 to 3 billion sperm commonly used in conventional AI. By this calculation, 400 to 500 doses could be potentially prepared from each ejaculate. With this technique it was possible to insert the insemination catheter past the cervix to deposit semen in the anterior region of one uterine horn in about 90% of the sows, with subsequent

farrowing rates and litter size of 85% and 9.5 piglets, respectively. Although endoscopic deep uterine insemination is an alternative technique for insemination of a small number of spermatozoa in sows, the flexible fiberoptic endoscope is an expensive and fragile instrument to be used under field conditions.[18] For this reason, a new catheter for insemination with flexibility and propulsion force similar to those of the fiberoptic endoscope was used in a subsequent trial.[19] Sows were inseminated within the anterior region of one uterine horn 36 hours after administration of human chorionic gonadotropin (hCG) with 150, 50, 25, or 10 million sperm cells in a total volume of 10 ml of Beltsville thawing solution (BTS) diluent. Investigators were able to inseminate and impregnate almost 90% of the sows, and no difference in litter size was observed. Only use of large doses of 50 million and 150 million sperm cells for deep insemination, however, resulted in pregnancy rates comparable with those achieved with insemination using a conventional dose of 3 billion sperm (74.4%, 80.9%, and 79.8% respectively). Apparently, by ligating the noninseminated horn, investigators were able to demonstrate that after deep insemination, sperm cells travel transperitoneally through the opposite infundibulum to fertilize oocytes ovulated from the contralateral ovary.

Several research institutions have experiments under way to validate low-dose, deep-uterine insemination techniques using different catheters for deposition of fresh, frozen-thawed semen with spermatozoa sorted for sex. In a recent commercially based field trial, a 200-mm catheter that extends over the insemination rod (Deepgoldenpig*) was used for deep insemination of sows with reduced sperm numbers.[20] During this trial, doses of 1, 2, or 3 billion total spermatozoa diluted in X-cell extender* () to a standard volume of 80 ml in a flatpack bag were used to evaluate the insemination device in more than 3000 sows. At the two higher sperm doses, no difference in farrowing rates was found between the experimental insemination device and a control catheter, and both gave rates exceeding 90%, with a mean litter size of 12.4. At the 1 billion sperm dose with the conventional catheter, however, the farrowing rate dropped to 66% with a litter size of 10.3, but with the Deepgoldenpig, the farrowing rate remained not significantly different from that in the other groups at 87% with a litter size of 12.1. Currently, use of this insemination technique device provides a method to reduce the insemination dose for sows without serious loss in production. Because of difficulties with catheter insertion and increased risk of reproductive trauma and hemorrhage, intrauterine insemination is not recommended for gilts. At present, several practical issues prevent its routine commercial acceptance for farm use. This technique requires trained inseminating staff and good estrus detection to avoid late (postovulatory) inseminations.

Low-dose insemination in combination with a single (fixed-time) insemination has the potential to give an even higher economic advantage in genetic dissemination, with doubling of current boar productivity. Owing to the nature of the estrous cycle in pigs, however, it will be very difficult to predict the right time to perform a single-dose insemination per cycle to achieve a farrowing rate and litter size comparable with those obtained with multiple inseminations. Traditionally, multiple (two or three) inseminations are performed throughout estrus to increase the chances that one insemination will be performed at an optimal time relative to ovulation. In recent years, the use of real-time ultrasonography (transabdominal or transrectal) has helped redefine insemination strategies by characterizing ovulation patterns in the sow retrospectively. As a consequence, optimal time for insemination has been found to be in the interval from 28 hours before to 4 hours after ovulation.[21] Ovulation in the pig normally occurs approximately 38 hours after the onset of estrus, but this timing can vary considerably between females (16 to 52 hours) and with genetic makeup and environment.[22] In addressing the topic of potentially reducing the number of inseminations and therewith allowing the possible use of fixed-time AI, it is essential to recognize that accurate heat detection and familiarity with general estrous behavior in gilts or sows are essential components to improve the prediction of timing of ovulation and for the implementation of an AI schedule. Although up-to-date ultrasonography does not appear to be effective in predicting time of ovulation,[23] defined relationships between the time of ovulation relative to estrous behavior have led to methods that allow producers to more accurately pinpoint the best time for insemination.[24]

SPERM SEXING TECHNOLIGY

Being able to preselect the sex of offspring at the time of conception ranks among the most sought-after reproductive technologies of all time. Sexing mammalian sperm has several important applications in the swine industry; however, sperm sexing will have a huge impact on the efficiency of existing multiplication systems. We are seeing dramatic changes in swine production with consolidation and vertical integration that affects how swine are produced and marketed. The use of sex preselection has great potential for increasing efficiency. In the future, those not taking advantage of efficiencies such as sex control may be buying animal products from those who do.[25]

For several years, investigators have attempted more than a dozen approaches to separate the X chromosome– and Y chromosome–bearing sperm based on perceived and assumed differences in physical characteristics or immunologic factors, with little success. Recently however, a new approach based on the well-established differences in DNA content between the two sex chromosomes has been the only consistently successful method of sorting sperm from a variety of species, including swine.[26] This DNA fluorescence–activated cell sorting technique involves treating sperm with a fluorescent dye, Hoechst 33342, which binds to their DNA, and then sorting them into separate X- and Y-bearing sperm populations using a flow cytometer–cell sorter modified

*IMV International, L'Aigle Cedex, France.

specifically for sperm to measure the relative fluorescent intensity reflective of DNA content of each sperm as it traverses a laser beam. Flow-cytometric separation of viable X chromosome– from Y-chromosome–bearing sperm is now a well-established technique and has proved to be repeatable at numerous locations and with many species at greater than 90% purity.[27] On average, the X-bearing sperm of common mammals contain 3% to 7% (3.6% in swine) more DNA than the Y-bearing sperm.[28] The wider the difference, the easier it is to sort with great accuracy.

Offspring of the predetermined sex in swine and other domestic animal species have been born after insemination using this technology since its introduction in 1989.[12] The current technology, however, has certain obstacles that prevent a broader application. First, the number of spermatozoa that can be separated per hour is low (about 16 million X-bearing sperm at 73% to 80% purity); second, labeling with the fluorochrome decreases sperm viability, leading to a higher embryonic mortality; and third, the expenses associated with use of an appropriate flow cytometer are enormously high. Nevertheless, new technological advances in high-speed cell sorting technology are being made to make the process produce more viable X and Y sperm per unit time at the highest purity.[27] Although the limited numbers of sperm available with flow-cytometric sperm sorting can be circumvented by using IVF or ICSI and subsequent embryo transfer, these are likely to be minor avenues for sex regulation relative to AI because semen can be delivered through different routes and techniques such as surgical (intrauterine or intratubal), deep uterine, cervical, and laparoscopic. In pigs, these sperm numbers are still insufficient for conventional insemination techniques; however, it recently has been shown that a numerical reduction in the number of sperm per insemination and the use of sorted sperm do not necessarily affect the pregnancy rates if semen is deposited surgically closer to the uterotubal junction a few hours before ovulation. It was demonstrated that the number of sperm inseminated can be reduced by several orders of magnitude in a low insemination volume down to 5×10^6 in 0.5 ml of Androhep extender per uterine horn in hormonally stimulated prepubertal gilts[29] or in sows[30] without significant reduction in rates of pregnancy or farrowing or in litter size.

Currently, sexing sperm is a tool that has matured sufficiently to be valuable for experimental purposes and for certain niche applications related to production animal agriculture. For wide agriculture applications, cost must be low and efficiency and convenience, high. Skewed sex ratios of 85% to 100% of one sex or the other have been repeatedly achieved in most species. In a preliminary experiment, pigs of the desired and predicted sex were born after surgical insemination at rates of 74% females and 68% males.[12] Offspring all were morphologically normal and were reproductively capable in adulthood, showing no negative effects of the sorting process. With improved techniques for pig IVF, sexed sperm were used to fertilize in vivo–matured oocytes, and the resulting embryos were used to produce two litters of pigs, all of the desired sex.[14] This was the first successful use of sexed sperm to produce offspring of preferred sex after transfer of sexed embryos derived by in vitro embryo production (IVP).

Improvements in IVF protocols led to more investigations in this field but now using ova matured in vitro. In one study, ova inseminations were done within 1 hour of completing the sorting process, and embryos were transferred at the four-cell stage.[31] Thirty-four piglets were born from 6 embryo recipient sows; 33 were females and 1 was a male (97% success rate). In another study, in vitro matured pig oocytes were fertilized with either X- or Y-sorted sperm at 20 hours after sorting.[32] Three litters were born from embryos produced from Y-sorted sperm, resulting in 9 male piglets (100%), and 5 litters were born from transferred embryos produced from X-sorted sperm, resulting in 23 females and 1 male (97%). Collectively, the results from these experiments demonstrated the effectiveness of using sexed sperm to produce sexed offspring in the pig by first producing sexed embryos. Currently, new flow cytometer machines are being perfected for commercial use through collaborations between the Beltsville Germplasm and Gamete Physiology laboratory and several scientists around the world.

Another approach for sperm sexing, currently under development, is based on the hypothesis that sex-specific proteins present on the surface of the sperm cells of a desired sex can be removed by polyclonal antibodies that, when added to the sperm, will result in agglutination of the cells. The free-swimming sperm cells can be then filtered off and used for fertilization of oocytes in vitro.[33] The preliminary results of trials conducted in bovines suggested that this viable immunologic sperm sexing procedure has potential for application in other species, including the pig.

EMBRYO TECHNOLOGY

Embryo Transfer Procedure

Strictly speaking, the term *embryo transfer technique* refers only to the process that begins with embryo collection and continues through embryo transfer. The basic technique of **embryo transfer** consists of removal of an embryo from a genetically superior animal and introduction of that embryo into a host surrogate. The resulting offspring will have qualities that duplicate those of the embryo supplier while not retaining any characteristics of the surrogate. Although embryo transfer in pig was first reported more than 50 years ago (see Table 112-1), it has limited applications in commercial swine production. In the swine industries, the primary reasons for recovering and transferring embryos are to minimize the risk of disease transmission, to propagate genetically superior animals, and, in some cases, to simplify shipment of genetic material. In addition, the application of embryo transfer technologies to breeding strategies also will generate significant advancements in selection, by allowing for the constant introduction of new genetic material into nucleus breeding herds. Embryo transfer provides a complete genetic package to the purchaser, resulting in rapid genetic upgrading at the lowest possible cost and lowest risk of disease transmission. Embryo transfer has been well established as an important tool in research,

and a great deal of current knowledge regarding reproductive physiology in swine has been a result of research studies using this technique. In practical application, embryo transfer comprises a complex series of measures including selection and stimulation of donor sows, recovery of embryos, embryo handling, and transfer of recovered embryos to recipients.

Superovulation and Donor Selection

The rationale for use of technologies increasing the number of embryos during collection is obvious for several domestic species. Gilts and sows, however, normally ovulate between 10 and 25 eggs, so superovulation has not been essential; accordingly, very few research groups have been interested in this development. Nevertheless, it is estimated that a total of 20 to 25 embryos per donor sow can be potentially collected, and use of superovulatory protocols apparently will increase the number of embryos collected per donor, thereby justifying the cost and time of the procedure. In the United States, no commercial product is currently available for induction of multiple ovulation in pigs, and porcine or ovine follicle-stimulating hormone (FSH) preparations are relatively ineffective in inducing superovulation in swine.

Several available gonadotropin preparations have been used in swine to increase the number of ovulated oocytes. Traditional methods of synchronization accompanied by superovulation include the use of 1000 to 1500 IU of equine chorionic gonadotropin (eCG) (e.g., Folligon* or Novormon†) either 24 hours after weaning in sows or 24 hours after a luteolytic dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (e.g., Lutalyse‡) administered on days 12 to 15 of the estrous cycle in mature gilts, or administered any time to prepubertal gilts. In cycling gilts, eCG also can be administered after an altrenogest (Regu-Mate*) feeding regimen providing 15 mg/day of altrenogest for 15 to 18 days for estrus synchronization. To induce ovulation, 500 to 750 IU of hCG (e.g., Chorulon*) also is administered 72 to 80 hours after eCG, and the animals are inseminated 36 hours after that. The response after such treatments can be highly variable, however, even in animals of the same weight and age. Factors such as product impurity due to luteinizing hormone (LH) content and lot-to-lot variation in bioactivity also may contribute to the variable response.

Using this hCG-eCG protocol, our laboratory has been able to obtain a mean of 23 ± 5.0 total ova or embryos per donor, compared with approximately 15.2 ± 3.8 from non-superovulated gilts. In a recent review of 12 studies using gilts as donors, however, investigators report superovulatory responses as high as 32 embryos collected, but in some cases no improvement in superovulatory response was observed.[34] In addition, with repeated embryo collections, more than one batch of embryos per donor also could be obtained. The methods of embryo collection will determine how often collection from one donor is possible. The stage at which collection takes place (days after mating) has an impact on the type of technology to use. For non-superovulated animals, pubertal gilts and sows can be used successfully as donors. In my experience, a higher embryo yield has been obtained in mature gilts (those undergoing more than three estrous cycles) and sows. More recently, investigators reported that very young prepubertal gilts (approximately 100 days of age) were poor candidates for embryo donors or embryo recipients. Lower pregnancy rates were achieved when these animals were used as embryo donors and lower pregnancy rates and retardation of fetal growth when they were used as embryo recipients.[35] It also has been reported that embryos from prepubertal gilts do not have the same in vitro developmental capacity as those from cyclic gilts when surgically collected at early stages of development and culture in vitro to the blastocyst stage.[36]

Embryo Collection Techniques

Embryo recovery in swine usually is accomplished surgically with the animal under general anesthesia, with the reproductive tract presented through a midventral incision in the caudal abdominal region involving retrograde flushing of the oviducts or uterine horns, or both. Surgery in swine is simple, inexpensive, and reliable once the necessary skills are developed. This surgical technique for embryo collection has been described previously and remains relatively unchanged.[37,38]

Other, less invasive alternative techniques for embryo collection also have been investigated. These techniques include nonsurgical embryo collection after surgical resection of the uterine horns[39,40]; endoscopic techniques[41,42]; and flushing of the reproductive tract after slaughter of the donor animal. Collectively, these techniques have some limitations, and results obtained with these procedures need to be improved. The number of ovulations, the age of the animal, the day of collection, the quality of the semen, and the fertility of the donor sow or gilt all influence the success of embryo collection. Results with traditional surgical embryo collection suggest that a success rate of about 90% recovery can be expected when an average group of donor gilts or sows is used.

Embryo Manipulation and Embryo Yield

After collection, the fluids are left to settle, to allow the embryos to sink in the bottom of the tube. Embryos are morphologically examined in vitro under a stereomicroscope and transferred into a holding or culture medium. Embryos also are evaluated according to their morphologic characteristics at the expected stage of development, and then viable (transferable) embryos are selected. Swine embryos usually are recovered over the first 5 to 6 days after the onset of estrus (about 120 hours after ovulation) to ensure that collection is performed while the embryos are still contained within the zona pellucida. At this stage, morula and early to expanded blastocysts generally are recovered. They also can be recovered at different stages of development, however. Embryos at the one-cell to the early four-cell stage are col-

*Intervet, Millsboro, Del.
†Vetrepharm, London, Ontario.
‡Pfizer Animal Health, Exton, Penn.

lected from the oviduct on days 1 to 3 after ovulation, whereas embryos from the four-cell stage up to the hatched blastocyst are flushed from the proximal uterine horn on days 4 to 7. Embryos with a difference of one cell cycle can be accepted as transferable, whereas embryos less developed could be left in culture for an additional 24 hours before transfer. Embryo evaluation 24 hours after culture gives a good indication of their developmental capacity, and embryos that do not develop after culture can be discarded.

In a study performing embryo collection in 229 non-superovulated donor gilts, our laboratory obtained a mean number of corpora lutea of 15.2 ± 3.8, a mean total number of ova or embryos of 13.4 ± 4.2, and a mean number of viable (transferable) embryos of 11.8 ± 4.8. These results translated to an 88.1% recovery rate and a proportion of transferable embryos of 86.0%. When gilts were grouped by the number of estruses detected (one to three or more) before embryo collection, however, a significant increase was demonstrated in the number of corpora lutea, total ova or embryos, and total number of transferable embryos as the number of estruses increased before collection.[43] By contrast, when embryos were collected from non-superovulated sows (greater than fourth parity), a higher number of transferable embryos (up to 20) was obtained.

Embryo Transfer

Selection of recipient gilts has a major impact on embryo transfer results. Although prepubertal gilts can be used as recipients, cycling gilts and sows are preferred because of their stable endocrine and uterine development. As in embryo transfer programs in cattle, management of the recipient probably is an important factor affecting pregnancy rate. Recipient animals, in which the embryos are to be implanted, are not inseminated. For embryo transplantation to be successful, however, it is necessary for the recipient animal to be in the same stage of the estrous cycle as the donor. The degree of synchrony between donor and recipient needed during a surgical embryo transfer is not as precise as in cattle. Traditionally, during a surgical embryo transfer, investigators prefer to transfer embryos into recipients at either the same stage of estrous cycle (day 0) as in the donor or into asynchronous recipients (–24 hours) with a less advanced uterine development. It has been previously demonstrated, however, that during a nonsurgical embryo transfer procedure, recipients that ovulated between 24 hours before and 12 hours after the donor achieved a higher pregnancy rate. This finding suggests that transfer to recipients ovulating later than 12 hours after the donor is associated with very low pregnancy rates.[44]

Although several groups of investigators are making progress in the area of nonsurgical embryo transfer to recipients, traditional surgical transfer is still the method of choice for most embryo transfer programs.[45] Surgical embryo transfer is carried out using a ventral midline laparotomy approach, after general anesthesia has been obtained. One of the ovaries and the adjacent proximal portion of the uterine horn are exteriorized from the surgical incision. After examination of the ovary for identification of a sufficient number of corpora lutea, the embryos are transferred, depending on their stage of development, either to the oviduct (one-cell to early four-cell embryos) or into the tip of the uterine horn (four-cell embryo to hatched blastocyst). Embryos are transferred in a small amount of medium by means of transfer pipettes or catheters.

Other procedures have been evolving for transferring embryos including transcervical (nonsurgical) techniques. For a long time, nonsurgical embryo transfer in pigs has been considered a technique that was almost impossible to perform. The reason was the complex nature of the genital tract with its constricted cervical canal and long, coiled uterine horns, as well as the location of the embryos in the proximal area of the horns during early pregnancy. These challenges have discouraged attempts at nonsurgical transcervical introduction of a catheter into the uterine horn. During the last decade, several experiments regarding nonsurgical procedures were reported in detail by five independent research groups, all using different approaches.[13,44,46–49] The three main differences among various procedures are the use of sedation, the type of instrument used (AI spirette or special design), and the volume of fluid used for transfer. Collectively, results from these studies have demonstrated that nonsurgical transfer of embryos in swine is feasible and has resulted in pregnancy rates ranging from 9% to as high as 64% and average litter sizes ranging from 3.1 to 10.9 piglets. Although all groups used different approaches for their transfers, the best pregnancy rates were achieved without recipient sedation.[44,49] On the other hand, specially designed instruments, which enable deposition of embryos into the uterus with a small volume of fluid, resulted in higher litter sizes (approximately 6 to 11 piglets).[44,47,48] More recently, the nonsurgical embryo transfer technique in sows developed by Hazeleger and Kemp has been applied under commercial farm conditions.[50] Nonsurgical transfers were performed in 45 synchronized recipient sows, each receiving 25 to 30 blastocyst-stage embryos. The farrowing rate (44%) and litter size (7.4 ± 3.2) obtained were lower than those achieved with the experimental conditions previously reported (59% and 10.9 ± 3.4, respectively).[44] In a subsequent small trial, a modified flexible catheter, similar to the ones used with a fiberoptic endoscope, was successfully deployed to penetrate the anterior third of one uterine horn during early diestrus.[51] With this procedure, a pregnancy rate and litter size of 72.7% (8/11) and 6.0 were obtained after transfer of 24 to 30 morula-to-blastocyst stage embryos, placed at a depth of 30 to 70 cm into the uterine horn in a small volume of medium (0.8 ml).

Endoscopic procedures for embryo transfer also have been recently developed.[42,52,53] This minimally invasive technique has some advantages, including repeatability compared with surgical procedures, but requires expensive endoscopic equipment and very experienced handling and usually takes longer than laparotomy procedures do. The equipment consists of a small cold-light fountain, a light cable, a metal catheter for an oblique optic telescope, a metal catheter for an atraumatic forceps, and a metal catheter for the venous catheter. Five cm caudal to the umbilicus the catheter for the optic is

introduced into the abdomen, and 10 cm lateral of the optic the catheter for the optic also is inserted. After observation of the ovary for the presence of corpora lutea, the forceps is used to stabilize the infundibulum and embryos are deposited by advancing the venous catheter. Using this technique, pregnancy rates greater than 40% and litter size ranging between 2 and 10 piglets have been reported.[54]

Regardless of the reproductive technologies used to propagate genetically superior animals, it must be recognized that the development of a nonsurgical embryo transfer method that is simple, rapid, and cost-effective will eventually lead to adoption of the technology. Although the results achieved to date are still lower than with the traditional surgical procedures, a commercially viable nonsurgical embryo transfer (NSET) technique will revolutionize the hog industry and ultimately alter the method of genetic dissemination in swine.

Pregnancy Rate after Embryo Transfer

Under optimal conditions, high-quality embryos with intact zona pellucida can be efficiently collected and transferred by established surgical procedures. The average pregnancy rate on transfer of embryos without an extended intermediate in vitro phase after collection reaches 80% to 85%, and approximately 35% to 50% of the transferred embryos survive to term, which is similar to the rate after AI insemination or mating.

Traditionally during surgical transfer, embryos are deposited in the tip of the horn distal to the uterotubal junction. Embryo deposition at this uterine site has achieved good pregnancy rates. By contrast, it was previously reported that when embryos were transferred to different sites along the uterine horn, embryo survival and pregnancy rate dropped dramatically (3% and 12%, respectively).[55] These results suggested that the uterine body seems to be an unsuitable site for embryo transfer in pigs and explained the unsatisfactory results that some investigators have achieved with nonsurgical embryo transfer. Unlike with surgical embryo transfer, during the nonsurgical embryo transfer procedure other factors, apart from uterine site deposition, also may be critical for a successful embryo survival. These factors, as previously mentioned, include the recipient's age and superovulatory program, size and stage of development of the embryo at the time of transfer, donor-recipient synchrony, number of embryos transferred, and volume of fluid during embryo transfer deposition.

Interest in porcine embryo transfer research is strong in several institutions. Published reports, however, demonstrated that when embryos are transferred surgically, pregnancy rate and litter sizes were significantly higher than those achieved with nonsurgical embryo transfer techniques. These results clearly demonstrate that several modifications are needed in order to improve results with nonsurgical embryo transfer.

The transfer of 16 to 22 embryos seems to be optimal to achieve high pregnancy rates, and the nonsurgical transfer of up to 30 and 40 embryos has been reported. Some investigators have been surgically transferring an average of 21.4 embryos, resulting in a 35% embryonic survival rate. When fewer embryos (about 16) are transferred, however, the embryonic survival rate increases to as high as 50%. Apparently, a strong positive correlation has not been found between the number of embryos transferred and the number of piglets obtained after parturition.[43]

Factors such as the superovulatory regimen, technique of collection and transfer, age of the donor and the recipient, embryo quality and stage of development, embryo culture, synchrony of the donor and the recipient, and management of the recipient affect the pregnancy rate after embryo transfer.

Embryo Storage

Porcine embryos typically are transferred within an hour of recovery or placed in culture at 39° C for up to 4 hours before being transferred. Embryo storage in culture medium for an extended period of time (up to 48 hours), during which the embryos continue to develop or are arrested at a certain stage of development, is an important consideration, however. The ability to support in vitro development of embryos is essential to biotechnological applications such as cloning and production of transgenic animals. Washed and certified embryos can then be transported to any destination across the globe in highly protected and reliable small shipping incubators.

The development and implementation of an extended embryo culture are obviously another important point to consider in an embryo transfer program, to enable transfer of embryos to recipient host sows anywhere in the world. Another advantage is greatly reduced shipping costs and potential losses in comparison with those for live animal transport. These combined economic and health advantages make embryo transfer a logical choice for the safe international exchange of swine genetic material.

It recently has been reported that in vitro culture up to the blastocyst stage of development of in vivo–fertilized embryos collected at early stages of development is very effective when North Carolina State Medium-23 (NCSU-23) or Beltsville Embryo Culture Medium-3 (BECM-3) is used.[36] Embryos also can be cultured for 24 hours in a simple TL Hepes medium, in which they can successfully grow to a more advanced stage of development.[56]

Until recently, only fresh porcine embryos could be transferred. Long-term preservation by freezing, however, is by far the most important technological development to ensure the application of embryo transfer in the swine industry. Since the mid-1980s, the animal industry has routinely used embryo freezing for extended preservation and storage of embryos of several livestock species, especially cattle. Unfortunately, because of physiologic and cellular differences in pig embryos, successful cryopreservation techniques have only been newly developed. Pig embryos can be severely damaged after exposure to hypothermic conditions (below 15° C). Because boar semen also is extremely sensitive to cooling and cryopreservation, however, this chilling sensitivity may be species specific. Although birth of live piglets after

cryopreservation has been reported, porcine embryos are limited in their ability to withstand freezing and cryosurvival.[10,11,57] This vulnerability is believed to be due, at least in part, to the high concentration of lipid material that pig embryos naturally possess. Presence of these lipid droplets may lead to uneven intracellular ice formation and is considered the major cause of embryo degeneration after freezing and thawing.[58] Subsequent experiments revealed that the freezability of pig blastocysts is greatest immediately after hatching at a size of 150 to 300 μm and then decreases rapidly when the blastocysts increase in size.[59] This finding suggests that older embryo stages contain less lipid material and subsequently have a greater tolerance to freezing. With regard to disease, however, protocols based on cryopreserved hatched blastocysts are not ideal, because the intact zona pellucida is an efficient barrier to various types of pathogens.

A new cryopreservation technique called **vitrification** is under development, with promising results. With this technique, embryos are equilibrated in a highly concentrated cryoprotectant solution for a few seconds and then frozen in liquid nitrogen. Rapid cooling prevents ice crystals from forming in and around the embryo, retaining many of the physical characteristics and ensuring a much more friendly environment for the embryo. Vitrification has been used in porcine embryos; however, only a few pregnancies have been achieved after this procedure. Early attempts to successfully cryopreserve pig embryos included micromanipulation to physically remove the intracellular lipids and resulted in the first litter born using this procedure.[15] Without such micromanipulation, pig embryos up to the morula stage do not survive cryopreservation.[60] Although piglets were produced, results with this technique for embryonic removal of cytoplasmic lipids before freezing were far from practical. Recently, investigators have been working on vitrification techniques that eliminate the need to remove the lipid material with some success.[61] Even more recently, however, using a modified vitrification technique, pig embryos were successfully cryopreserved without the need of micromanipulation.[62] For this procedure, intact morula or early blastocyst stage pig embryos were centrifuged and then vitrified. After thawing, the embryos were surgically transferred to asynchronous (–24) recipient females. This novel methodology resulted in a farrowing rate (82%) and litter size (7.0 piglets) comparable with those achieved with traditional surgical transfer of fresh porcine embryos. Results from this study clearly indicate that this method is both simple and inexpensive and has the potential for immediate commercial application. Although some fine-tuning of the procedure and demonstration of repeatability are required, this recent advance in methodology for freezing pig embryos will have major ramifications for the long-term preservation and movement of genetic material in the swine industry.

Elimination of Disease by Embryo Transfer Technologies

Because disease transmission is an important consideration in the swine industry, the possibility of transmitting infectious organisms through embryo transfer has been the subject of extensive investigation. Embryos are considered to be the safest agent for domestic or international exchange of livestock genetics, however. The disease status of the donor sow has no impact on the disease status of piglets derived from embryo transfer if proper embryo washing procedures are adhered to during the transfer process. This lack of health status relationship between donor sows and their offspring derived from embryo transfer arises from the protective barrier formed by the zona pellucida (eggshell). The zona pellucida completely encases the developing embryo over the first 6 to 7 days of life, creating an impermeable physical barrier to all disease-causing viruses and bacteria. Because bacteria and viruses cannot pass through this natural barrier to infect at the stage of transfer, the risk of disease transmission is reduced to its lowest possible level. Because of the impermeable nature of the zona pellucida, only those pathogens that can adhere to the outside surface of the zona pellucida could be transmitted with the embryo during the transfer process. Accordingly, washing the embryos after they have been flushed from the donor uterus is a very effective means of the elimination of those pathogens. In fact, provided that the zona pellucida is intact, the embryo washing procedures established by the International Embryo Transfer Society virtually eliminate any chance of pathogen transfer during embryo transplantation. With more than 20 years of use of this technique in the dairy industry, not a single case of disease transmission has been linked to the commercial transfer of embryos.

Because swine embryos can be certified pathogen-free after washing, breeders will be free to import new genetic stock without risk to the resident herd. This will allow internationally based breeding companies to rapidly, economically, and safely disseminate new genetic lines of breeding stock throughout their entire international network in a single step. In addition, embryo transfer can be used to "clean" valuable genetic lines of swine that have become infected with disease-causing pathogens. Embryos recovered from infected donors can be washed and transferred to clean recipients, thereby breaking the disease cycle and producing pathogen-free offspring that nevertheless retain the full genetic composition of the donor line. With all of this knowledge, however, only a few reports have described any extensive use of embryo transfer in minimizing the risk of disease transmission. James and associates[5] used embryo transfer to successfully conserve valuable genetic material from pseudorabies-infected swineherds in the United States. In another trial, investigators used embryo transfer to introduce newly imported genetic material into five specific pathogen–free (SPF) herds in Australia.[63]

More recently, confirming results of previous trials, researchers used embryo transfer to successfully conserve valuable genetics from porcine reproductive and respiratory syndrome virus (PRRSV)-infected herds.[43] Experimentally, several investigators had reported the use of specific embryo washing procedures to eliminate pathogens from embryos collected from donors infected with PRRSV,[64–66] hog cholera virus,[67] and many other viruses.[68] Collectively, results from these studies have

demonstrated that embryo transfer will result in the birth of virus-free piglets provided that the embryos are washed (or unwashed, as in one report of PRRSV elimination) and pathogens were not detected or were not present during the early stages of development. These findings indicate that this technique is a highly effective clinical tool for the elimination of pathogens from an infected breeding stock.

IN VITRO EMBRYO PRODUCTION

For many years, scientific interest has existed in the capture of genetic material present in the ovaries of superior sows, in a manner similar to the use of AI for the boar. **In vitro embryo production** (IVP) is a collective term used to describe the three steps in the in vitro process in which recovered immature oocytes are matured (in vitro maturation [IVM]) and fertilized (IVF) in the laboratory, with the resulting embryos subsequently cultured in vitro (in vitro culture [IVC]) to a transferable stage of development. IVP of swine embryos has been one of the more rapid technological advances of this decade. The difference that gives IVP an advantage over embryo transfer from a genetic or breeding standpoint is the potential for acquiring a large number of oocytes from a given animal. The number of oocytes acquired could be considerably greater than the maximum number of in vivo embryos that can be obtained. It was estimated that a female pig may have approximately 500,000 or more primordial follicles containing oocytes stored in her ovaries at the time of birth. These stored oocytes will be all she will ever produce in her lifetime and will develop or regress during the course of her productive life. Success in IVP has stimulated increased research in other areas that can be enhanced by the availability of embryos without requirement of surgical collection from gilts or sows. One example is the combined use of IVF, sperm that have been sorted for sex, and embryo transfer to produce offspring of a predicted sex.

In vitro embryo production is a multi-step process that requires a well-equipped laboratory and a skilled technician. The IVP process involves harvesting immature oocytes from the ovaries and maturing them for about 44 hours in either tissue culture medium (TCM) 199 or in NCSU-23. To induce oocyte maturation, the culture medium is supplemented with hormones, macromolecules (follicular fluid, fetal calf serum [FCS], bovine serum albumin [BSA], or polyvinyl alcohol [PVA]), and growth factors. Matured oocytes are then fertilized in vitro, and the resulting embryos are held in culture medium inside an incubator (at 39°C and 5% CO_2 in humidified air) for 6 additional days and then transferred to an unmated recipient female at the same stage of the estrous cycle as for the donor female. The pregnancy success rate for good-quality IVP-derived porcine embryos is expected to range from 50% to 75%.

Early studies on IVM clarified the importance of both nuclear and cytoplasmic maturation on the developmental competence. In a preliminary report, when porcine follicular fluid (pFF) and FSH were added to the maturation medium, nuclear maturation to metaphase II was enhanced and blastocyst formation (29.2%) was superior compared with maturation rates without pFF (11.5%) or FSH (8.7%) after IVF.[69] The results of this study clearly indicate the beneficial effects of pFF and FSH on the maturation process and overall embryo yield in vitro. Although some investigators have reported pregnancies and live piglets after transfer of IVP porcine embryos, the developmental potential of in vitro–produced embryos is still lower than that of their in vivo counterparts.[70–72] Cheng and associates reported production of the first piglets through IVF in 1986.[8] In vitro embryo production techniques, however, have not been developed for swine to a commercially usable state, compared with this technology for other domestic species. Numerous reports now testify to the fact that little or no difference exists between in vivo–and in vitro–derived cattle embryos subjected to transfer in terms of ability of the mature animal to produce calves. By contrast, very few reports exist demonstrating that normal offspring can be produced from the transfer of in vitro–derived swine embryos. One of these reports described a recent study aimed at improving the developmental competence of pig oocytes.[72] The investigators reported no differences in blastocyst development between oocytes matured in a protein-free medium supplemented with epidermal growth factor (37%) and those matured in a control medium (NCSU-23 plus 10% pFF) (also 37%), and a farrowing rate of 72% was obtained after embryo transfer to unmated recipient animals.

Analysis of published reports suggests that several obstacles in IVP of porcine embryos need to be overcome before such techniques become commercially available. One of the limitations to practical application of IVP technology in swine is the high incidence of multiple sperm penetration occurring during the IVF process. **Polyspermy** is the term used to describe the occurrence of oocyte penetration by more than one or two sperm cells. Polyspermy in swine oocytes ranges in frequency between 13% and 90% and is associated with formation of an irregular male pronucleus and perturbation in early embryonic development. Under in vivo conditions, fertilization occurs in a few hours after ovulation, and the female reproductive tract evidently plays an important role in regulating the initial number of spermatozoa that reach the site of fertilization to obtain, in most instances, monospermic penetration.[73]

In order to improve monospermic penetration and cleavage rates during the IVF process, investigators have to either modify the fertilization media components or reduce the number of sperm cells per oocyte without affecting the penetration rate. One possible strategy for reducing the occurrence of polyspermic penetration is to fertilize oocytes in an oviductal epithelial cell monolayer[74] or in the presence of oviductal fluid collected from gilts during estrus.[75] A low rate of polyspermy, however, also is accompanied by reduced sperm penetration.[76] In an experiment in which the concentrations of Ca^{2+}, lactate, and PO_4^- were different in the fertilization medium, no differences were observed in monospermic penetration.[71] Many research laboratories produce in vitro pig embryos developed from oocytes aspirated from prepubertal gilts after slaughter. More recently, however,

investigators were able to demonstrate that when compared with those from adult animals, oocytes aspirated from ovaries of prepubertal gilts have an increased incidence of polyspermic fertilization.[77] This finding suggests that adult ovaries contain oocytes with a higher developmental competence that that of prepubertal ones. Clear developmental difference also has been observed after IVF of pig oocytes, and investigators have demonstrated that oocytes matured in vivo displayed higher penetration and cleavage rates, a synchronous pronucleus development, and also a lower polyspermic rate compared with oocytes matured in vitro.[78]

Another obstacle limiting the potential practical application of IVP technology in swine is that currently this technology depends on a supply of developmentally competent oocytes from large antral or preovulatory follicles, which are present in the ovary of prepubertal gilts in only relatively small numbers (two to six) at the time of slaughter and aspiration. These ovaries, however, contain a number of primordial and preantral follicles that potentially can be harvested and cultured in vitro to a mature stage of development. Studies involving isolation of immature ovarian follicles and analysis of the requirements, metabolism, and differentiation processes of IVM of ovarian follicles already have been undertaken by researchers using ovaries from prepubertal gilts or any of several other animal species. Unfortunately, after several years of investigation, results with the culture–to–metaphase stage of development have been very disappointing. More recently, however, a study was conducted to identify an in vitro culture system that would support intact porcine follicle growth from preantral to antral stages all the way to oocyte maturation, fertilization, and embryonic development.[79] After 4 days in culture, 51% of these preantral follicles completed meiotic maturation to the metaphase II stage, and after fertilization, 13% of the cleaved zygotes developed to the blastocyst stage.

Oocyte Collection

Oocytes can readily be aspirated either from ovaries collected at slaughter or after a valuable female dies or undergoes ovariectomy. To preserve the genetic material and obtain a higher number of oocytes from a desired superior animal, however, noninvasive techniques need to be developed in swine. Early attempts at retrieving oocytes from potential donors included surgical and less invasive laparoscopic procedures, but the number of procedures that can be performed safely without causing injury to the donor animal is limited.

In the mid 1980s, a method was developed in humans for retrieving oocytes using ultrasonography to visualize the ovary while a needle was guided transvaginally into the follicle.[80] These efforts paved the way for the new reproductive technology developed to harvest oocytes from live cattle.[81–83] Ultrasound-guided **transvaginal follicular aspiration**, also known as ovum pick-up (OPU) in human medicine, recently has come under evaluation in pigs and other domestic and exotic species. Ultrasound-guided transvaginal follicular aspiration combined with embryo IVP is emerging as a successful method for obtaining preimplantation stage embryos. This nonsurgical method has become a common procedure for repeated retrieval of immature oocytes from ovarian follicles in live animals and has the potential for replacing previous procedures of conventional superovulation and traditional embryo transfer technologies. The prime advantage of this procedure resides in the fact that immature oocytes potentially can be aspirated in a continuous basis from follicles of mature sows, and possibly in prepubertal gilts as well. Oocytes also can be collected mature from live donors, thereby eliminating the need for IVM, or can be potentially collected from pregnant donors or a few days after weaning. In addition, this approach provides a highly efficient source of oocytes for the production of embryos for research purposes or for certain commercial applications that require zygote or embryo manipulation. Transvaginal follicular aspiration has the additional advantages of being noninvasive, with few risks to the animal, and being readily adaptable to on-farm application. Results from a preliminary study reported that the transvaginal ultrasonography–guided oocyte aspiration technique was successfully used to harvest oocytes from mature adult sows.[84] Using this aspiration procedure, 15 of 25 sows (60%) yielded an average of 4.5 oocytes/donor and had a mean of 6 follicles punctured/ovary. These findings indicate that this procedure has the potential for practical use in the future for IVP and nuclear transfer technologies. More research is needed to improve the efficiency of this approach, however. The oocytes were recovered from the ovaries of properly restrained donor sows by inserting a probe-guided stainless steel 55-cm, 17-gauge aspiration needle through the anterior vaginal wall near the cervix to aspirate the oocytes from the follicles detected by ultrasonography. A regulated vacuum pump produced negative pressure that caused the collapse of the follicle and allowed retrieval of the follicular fluid. The oocytes were harvested in phosphate-buffered saline (PBS) supplemented with 1% calf serum, 2 U of heparin, and 100 µg streptomycin/ml of medium. The follicles punctured were 4 to 6 mm in diameter, with more oocytes being produced from eCG-stimulated sows. All oocytes were morphologically evaluated, and 53% were classified as of good quality.

Intracytoplasmic Sperm Injection Procedure

Advanced techniques developed in conjunction with IVP include complex micromanipulation procedures such as injecting sperm into a matured oocyte (i.e., ICSI). Recent advances in ICSI have provided exciting opportunities not only for the treatment of male infertility but also for the study of gamete physiology during fertilization and early development. More recently, such a procedure has been studied for the production of transgenic animals using spermatozoa as a vector for introducing recombinant DNA into the oocyte. Although normal fertilization can be achieved by the injection of spermatozoa, sperm heads, or round spermatids into matured oocytes, the fertilization process after injection remained largely unknown. Despite the fact that human IVF using ICSI is relatively common and efficient, only a few offspring have been produced in laboratory animals and some domestic species including cats, cattle, and sheep.

Likewise, ICSI of porcine oocytes has been developing slowly, and it was not until recently that the birth of a single piglet was reported.[16] During this trial, in vitro–matured oocytes were injected with in vitro–capacitated spermatozoa; after 40 hours of culture, two- to four-cell-stage embryos were transferred to the oviduct of induced-estrus gilts 24 hours after the expected ovulation time. Pregnancy of the surrogate mothers was maintained using 5 mg of estradiol benzoate from days 10 to 16 after transfer to induce maintenance of the corpora lutea. Only one of four recipients carried the pregnancy to term; she gave birth to a single male piglet after receiving 32 embryos. Before this success, ICSI was performed in several studies using porcine in vivo–matured oocytes that were fertilized using either fresh ejaculated[85] or cytometrically sorted sperm[86] with good cleavage rate. All attempts to transfer cleavage embryos failed to deliver pregnancies.

Recently, experiments have been conducted to determinate fertilization rates and developmental ability of porcine oocytes after ICSI. In vitro–matured oocytes were injected with either spermatozoa, isolated sperm heads, round spermatids, or spermatid nuclei.[87] At 6 days after injection, the percentage of embryos developed in vitro to the blastocyst stage were 38% and 22% after ICSI and isolated sperm head injection, respectively. Unlike spermatozoa, spermatids are unable to activate oocytes, mainly because of the lack of activation factors. When electrical stimulation was used 2 hours before spermatid injection to induce activation, however, rates of formation of two pronuclei and blastocysts were not different in oocytes after intracytoplasmic spermatid and spermatid nucleus injection (25% and 27%, respectively). Results from these studies clearly suggest that although the embryos produced were not transferred, pregnancies and piglets could be produced after intracytoplasmic sperm or spermatid injection into matured oocytes.

GAMETE INTRAFALLOPIAN TRANSFER PROCEDURE

In an effort to circumvent some of the problems encountered with classic IVF techniques, swine reproductive researchers have recently turned to the technique of **gamete intrafallopian tube transfer** (GIFT), or oocyte transfer. This assisted reproductive technique is an alternative tool to produce pregnancies from genetically superior individual animals. The GIFT procedure is currently being used by several commercial embryo transfer centers in some domestic species, and pregnancy results are likely to improve. For this procedure, in vivo– or in vitro–matured oocytes collected from a live donor animal or at slaughter are transferred into the oviducts of a number of synchronized preovulatory recipients. At the time of donor oocyte transfer, all of the recipient's preovulatory follicles are aspirated to remove the "native" oocytes, and sperm are placed within the recipient's uterine or oviduct lumen, along with the donor oocytes. Removal of "native" oocytes from the recipient's preovulatory follicles will lead to luteinization and the development of functional corpora lutea. In swine, oocyte transfer would function in an equivalent fashion to that of embryo transfer as used currently, with the principal difference residing in the fact that the oocyte replaces the embryo as the primary unit of exchange. It is this difference that gives gamete transfer its primary advantage over embryo transfer from a genetic or breeding standpoint, in that the ability of today's veterinary scientists to reliably acquire large numbers of oocytes from every gilt being slaughtered is considerably greater than their ability to recover an equivalent number of embryos. Preliminary results indicated that the GIFT procedure can be used successfully in pigs and can be a valuable tool for the study of gamete interaction, as well as for the continued development of biotechnological procedures such as sex preselection.[88]

An alternative to this technique is the so-called **zygote intrafallopian tube transfer** (ZIFT) procedure whereby cleaved in vitro– or in vivo–matured and fertilized embryos are transferred to the oviduct of nonbreed recipients. Laparotomy traditionally has been performed for the oocyte or zygote transfer during these procedures in pigs. Of course, laparoscopy also could be an alternative to performing these procedures; however, reports have yet to be published regarding use of this technique in the pig.

PRODUCTION OF IDENTICAL MULTIPLETS

Many examples of cloning are found in nature. For instance, in human and animal reproduction, clones, or genetically identical animals, are produced when early embryos split into halves, creating identical twins. Each half contains the same genetic code. In the laboratory, artificial embryo manipulation attempts to achieve the same results through a variety of techniques including embryo bisection, dissociation of blastomeres, and cloning by nuclear transfer. A **clone** is a genetic copy of another living organism. Cloned organisms are produced asexually; sperm does not fertilize the oocyte. Hence, the genetic material of cloned offspring is drawn from a single source, rather than being a combination of sperm and oocyte genes.

Embryo Bisection

Creation of identical twins has been simulated in the laboratory by cutting embryos in half using different microtools. Embryo bisection, to propagate specific animals at a more rapid and efficient rate, is making dramatic progress in the field of embryo transfer technology. Monozygotic twin piglets also can serve as excellent models in various areas of biologic research. Bisection of bovine embryos has been applied widely under experimental and commercial conditions, and pregnancy rates after nonsurgical transfer of demi-embryos are only about 5% to 10% lower than those with nonbisected embryos.[89] In pigs, however, splitting of preimplantation stage embryos is associated with a significant decrease in viability in vivo, and very few sets of identical twin piglets have been obtained after transfer.[90–92] Several factors with profound effects on the viability of pig demi-embryos have been identified, such as the presence or absence of the zona pellucida, embryonic stage,[90,92] competition

between demi-embryos and intact embryos,[91] and temperature during the bisection procedure,[93] as well as experimental day, donor animal, boar, embryo quality, duration of the in vitro culture before bisection, and size of the halves after bisection.[94] Demi-embryos derived after splitting fair-quality blastocysts have a higher percentage of development in vitro (32%) than was observed for their morula stage (22.2%) or early blastocyst stage (16.7%) counterparts after bisection.[95] Results from a previous investigation demonstrated that pig demi-embryos possess a great developmental potential in vitro (66% were considered transferable after 24 hours in culture), and with transfer of a sufficient number of demi-embryos (about 28) per recipient, high pregnancy rates can be obtained (approximately 80%).[92] Although farrowing rate also was similar to that after transfer of intact embryos or after mating, litter size and postnatal survival were considerably reduced (6.0 piglets and 73%, respectively). These results suggest that in contrast with in vitro development, when demi-embryos are transferred to female recipients a significant reduction in developmental potential is observed. Nevertheless, higher success rates can be expected when embryos at the blastocyst stage with excellent morphologic quality are split into two even halves immediately after recovery.

Proliferation of Isolated Blastomeres

Use of isolated blastomeres derived from mammalian embryos has gained particular significance for purposes such as multiplication of genotypes from superior animals[96] or screening for sex determination, or for the transmission of genetic disorders.[97] In a preliminary report, approximately one third of blastomeres derived from four- to six-cell pig embryos developed to blastocysts after transfer to recipient gilts.[98] Several other recent experiments, however, demonstrated an improvement in developmental rate of isolated blastomeres after culture. Approximately 40% to 70% of single blastomeres derived from four- and eight-cell embryos could develop to normal morula and blastocysts after either a reaggregation of isolated blastomeres (transferred to a surrogate zona pellucida),[99] by being subjected to an electrical pulse to stimulate their development[100] or by modifications in the in vitro culture conditions.[101] Collectively, these results suggest that it is feasible to use dissociated blastomeres from embryos, at least up to the eight-cell stage, to produce a group of identical piglets after transfer.

Cloning by Nuclear Transfer and Gene Transfer in Pigs

Researchers have long been interested in nuclear transplantation both as a tool to study developmental biology and as a means for producing identical animals. **Nuclear transfer** is a term used to describe the basic technique that involves the transfer of a nucleus from one cell to another cell from which the nucleus has been removed. For cloning animals, this entails transferring the nucleus of a cell obtained from the individual to be cloned into a recipient oocyte that has had its own nucleus removed.[102] After transfer, the plasma membranes of the oocyte and donor cell are closely aligned so that they can be fused with an electric current to create a single cell. If the fusion process is successful, this newly created, single-cell embryo will begin to divide, becoming an embryo genetically identical to that of the donor from which the cell was obtained. This embryo can then be transferred into a surrogate mother for gestation.

Although the history of animal cloning dates back to the early 1900s, the most acclaimed example of animal cloning is, of course, the report by Wilmut and colleagues in 1997—the first to demonstrate that cloning of adult animals was possible.[103] Nuclei of cultured mammary epithelial cells derived from an adult ewe were transferred into enucleated sheep ova, ultimately resulting in the birth of a cloned lamb, Dolly. The first cloned sheep actually were described more than 10 years earlier by Willadsen, who used embryonic blastomeres as nuclei donors.[104] This was the precursor for subsequent work involving nuclear transplantation in domestic species and sparked considerable interest in the development and improvement of techniques for cloning livestock species. The impact of this work now seems minor compared with the explosion in research efforts targeted at cloning mammals that has occurred since Dolly's birth.

Without a doubt, one of the major factors influencing the probability of cloning a specific animal is species. Although the basic approach involving nuclear transfer may be similar, the specific material and methods utilized for cloning one species of animal do not automatically apply across different species. Cloning animals by nuclear transplantation involves several key steps, including (1) acquisition of mature ova, (2) removing the chromosomes contained within the ova (enucleation), (3) transfer of cell nuclei obtained from the animal to be cloned into the enucleated ova, (4) activation of the newly formed embryo to initiate embryonic development, (5) embryo culture in vitro, and (6) transfer of the cloned embryo into a surrogate recipient.

Progress in animal cloning technology is being fueled by the many potential benefits for humankind. Currently, efforts are focused on the production of transgenic animals by utilizing genotypes defined by a particular genetic modification to produce bioengineered pharmaceuticals, such as the production of proteins for the treatment of human diseases[105]; to increase disease resistance, growth, and reproductive performance in swine[106]; and to produce pig organs for transplantation.[107]A considerable demand also exists, however, for cloning animals with inherent genetic value. The production of recombinant pharmaceutical proteins is targeted to those expressed almost exclusively in the mammary gland or blood of transgenic pigs. In the past, genetic modifications (transgenics) of livestock could be achieved only by microinjection of several hundred copies of a specific gene construct into the pronuclei of a newly fertilized oocyte.[94] Currently, however, the use of cultured specific cells (with the desired genetic change) for genetic modification by nuclear transfer has several advantages in comparison with the method of direct injection. Although many transgenic pigs already have been produced using different DNA constructs by pronuclear microinjection, several

investigators are now, for practical reasons, applying nuclear transfer techniques for genetic modifications.[94]

The production of transgenic pigs by nuclear transfer for xenografts offers the best approach to creating organs for human transplants because of the similarities in size and biology. **Xenotransplantation** refers to the transplantation of organs or tissues from an animal of one species into another species (e.g., pig-to-human). The major limiting factor in organ transplantation today is the increasing shortage of suitable donor organs. It has been estimated that in the United States alone, approximately 60,000 people are listed for solid organ transplantation, yet only approximately one third of needed donor organs are obtained.[108] The discrepancy between the number of potential recipients and donor organs is increasing by approximately 10% to 15% annually; pigs, however, will hold promise for supplying an unlimited number of organs to replace ailing hearts, livers, and kidneys in humans. Pig organs, when transplanted to humans or nonhuman primates, are rejected hyperacutely within minutes, however, by antibody-mediated complement activation.[109] Methods have been successfully developed to prevent this hyperacute rejection, including depletion or inhibition of recipient antibodies or complement and the development of transgenic pigs that express a human complement-regulatory protein. Although experimental pig-to-primate organ xenotransplantation now results in transplant function for days and weeks, rather than minutes, the presence or return of anti-pig antibodies, however, even after the use of organs with the human protein, inevitably leads to what has been variously termed *acute vascular rejection* and *delayed xenograft rejection,* which is again believed to be antibody-dependent. The next step in pig cloning will be to modify the cells from which the pigs are cloned to prevent the human immune system from launching an acute rejection. Considerable progress has been made in recent years, but experimental results do not yet warrant the initiation of a clinical trial of organ xenotransplantation. It may take several generations of clones and many years to weed out the genes that cause rejection. Nevertheless, trials of pig cell transplants are already under way in patients with diabetes and neurodegenerative conditions, such as Parkinson's disease. Some researchers are concerned that using pig organs or tissue for transplantation could cause humans to contract some unusual swine viruses. These viruses have been infecting pigs for thousands of generations and now are part of the swine genes, passed along from parents to offspring.[110]

Animal cloning by nuclear transplantation is extremely inefficient, and in pigs only approximately 1% of the embryos transferred survive to term—even discounting the numerous trials that have resulted in no offspring. As mentioned before, pigs are a particularly illustrative example of a species in which techniques for nuclear transfer cannot be directly applied to other species. As with the sheep, the birth of cloned pigs also was reported first by investigators using embryonic blastomeres[9] and, a little more than 10 years later, by workers starting with somatic cells from an adult pig[17] using a modified double nuclear transfer technique, and then with fetal fibroblasts as nuclear donors.[111]

The first piglet to be born in these studies started as 1 of 88 reconstituted embryos that had been transferred, together with control embryos to increase the likelihood of maintenance of pregnancy. During the double nuclear transfer procedure, in vitro–matured oocytes were collected, enucleated, and fused with adult granulosa cells. The following day, a second round of nuclear transfer was performed by replacing the two pronuclei of in vivo–derived pig zygotes with the pseudo-pronuclei obtained from the nuclear transfer embryos. Embryos were then transferred into the oviduct of recipient females within 2 hours of the second nuclear transfer procedure. In total, 401 double nuclear transfer embryos were transferred into seven recipients, and 185 single nuclear transfer embryos were transferred into three recipients. Only one recipient receiving double nuclear transfer embryos became pregnant, and this animal gave birth to five piglets, whose birth was reported in *Nature*. In a second report, Japanese and British scientists each have claimed success in cloning pigs.[111] The Japanese piglet has been called Xena because of the belief by her creators at the National Institute of Animal Industry that she will contribute to the use of pigs as sources of organs for human transplantation. Fetal fibroblast nuclei were microinjected into in vivo–matured oocytes from which the nucleus had been removed. An electrical pulse stimulated the oocytes to grow into an embryo, and the resulting embryos were transferred into the oviduct of recipient females after a short-term culture. The transfer of 110 cloned embryos into four recipients resulted in the birth of one female piglet, whose birth is reported in *Science*.

The goal of current research is to grow thousands of cells in the laboratory, each of which will have a chance of developing into an entire animal once transferred into an oocyte. By applying transgenic techniques to thousands of cells in culture, those cells with appropriately altered DNA can be selected for nuclear transfer; then, if this procedure is successful, transgenic animals can be produced effectively. These made-to-order animals that possess new genes of special interest are the main focus of current research in cloning technology.

CONCLUSIONS

Reproductive technologies, such as embryo transfer, that allow for the simultaneous control and manipulation of both the genetic composition and the health status of selected animals will become an indispensable tool for internationally based swine-breeding companies. This methodology, combined with sperm sexing and deep uterine, single-low-dose insemination, may further reduce the overhead for producing replacement females. Nonsurgical methods must continue to be explored for embryo transfer before it will be widely applied in the industry. Potentially, embryo transfer could become a technique nearly as routine as AI. The use of these reproductive technologies will lead ultimately to the rapid development of genetic lines of swine specifically selected to meet the requirements of the markets in animal agriculture. In the past decade, amazing progress has been made with in vitro embryo production, nuclear transfer,

and related technologies. As they are refined, these technologies will no doubt be used increasingly, and entirely new areas for commercial applications will be discovered. Overall, the importance of animal health to the efficiency and profitability of the global swine industry may lead to the integrated use of reproductive technologies in a manner and level unmatched for any other livestock species.

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CHAPTER 113

Demographics and Herd Management Practices in South America

JULIO SUMAR

EARLY HISTORY

Current archeological evidence indicates that llamas and alpacas were domesticated in the Andean *puna* at elevations of 4000 to 4900 m above sea level.[1] Identification of the ancestral forms from which they were domesticated, however, remains a matter of debate. Once these animals were domesticated, llama and alpaca herding economies spread beyond the puna limits and became important for Andean peoples living in all geographic regions, from sea level to high mountain elevations.[2]

Everywhere in the Andean mountains, as well in lower-elevation sites and caves, rupestrian paintings more than 9000 years old have been found depicting camelids being herded or hunted. When the Spanish conquistadors arrived in the Andes in the early 16th century, they found millions of alpacas and llamas, together with a similar number of the wild species vicuñas and guanacos. They were distributed throughout the 380,000 square miles of the Inca Empire, from the coastal deserts to the snow line at 4500 meters above sea level. The culturally advanced Inca Empire relied extensively on the alpaca for fiber production and on the llama as a beast of burden and meat production, and used the wild species in a sound and rational manner. They also used these animal species as sacrificial animals and regarded them as tokens of wealth.

THE PLACE OF ALPACAS AND LLAMAS IN THE ANDEAN ECOSYSTEM AND IN THE ANDEAN CULTURE

Since their domestication alpacas and llamas have played a critical role in the Andean agroecosystem. In the cold Andean highlands, as well as along parts of the Pacific Coast, the **alpaca** is the most important source of wool. The **llama,** also a secondary provider of wool, became a most efficient pack animal, enabling the transportation of goods over long distances. As remains true today in traditional villages, both species were a very important source of manure for agriculture, which also served as fuel in the high treeless *puna* environment. Although meat has always been considered of secondary importance in the Andean diet, salted and freeze-dried camelid meat is a potentially important source of high-quality protein.[3]

Camelid bones provide the raw material for work utensils and beautifully crafted items, whereas sinews are turned into thongs. Camelid lard, in addition to acknowledged health properties in the rich Andean medical lore, still plays an important role in religious rituals. Camelid fetuses resulting from natural abortion are still sold in rural markets and are widely used in fertility rites. Similarly, stone formation in the digestive system of alpacas and llamas—**bezoars**—are considered to be charms possessing medical and magical properties.[3]

Finally, and not least important, alpacas and llamas have always been an abundant source of images and concepts in the rich and metaphorical Andean ancestral mythology. Herders state that "we take care of our animals and they take care of us,"[4] showing the strong interdependence of animals and humans. Peasants believe that *pachamama* (mother earth) gave alpacas and llamas to men as a loan, and that the future of humanity depends on the proper conservation of herds. They believe camelids originated in the underworld and came out from water springs. At the end of the world they will all return to those sacred springs. A sign that the end of the world is approaching, they say, will be depletion of the alpacas and llamas.[3]

Myth and rite also play an important role in enhancing animal fertility through propitiatory ceremonies that are part of the activities associated with mating, and with controlling herd size through animal sacrifice during the dry season.

Habitat

The continent of South America comprises 13 separate countries or territories and is the third largest land mass in the world. It contains the widest extremes of topography and climate and features the traditional and modern life and customs of many different sectors of human culture.

Along the western border of South America runs the high mountain range known as the **Andes,** or *Cordillera de los Andes*. These mountains are relatively young and are still in the process of being formed from the interaction of the South American and Pacific tectonic plates. Massive uplift has led to high, undulating land surfaces that contrast with the more continuous sharp relief of many other mountain systems.[5]

Faulting, folding, and volcanic activity have produced a rugged and mountainous surface topography, and glaciation and erosion have generated many deep valleys. The complex geologic history of the Andes has produced a landscape of rolling, relatively flat plateaus, with occasional mountain chains rising above them (approximately 6000 m above sea level) and deeply incised gorges cutting into them.[5] The term *puna* is used to refer to this intermediate zone, ranging in altitude from 3700 to 4800 m above sea level.[6] It has a relatively even terrain and its vegetation is characterized by bunch grasses and very sparse shrubs. It is this zone that constitutes the present-day habitat of the South American alpacas and llamas.

Two general climatologic seasons occur in this part of the Andean region: a mildly warm, rainy growing period from December to April and a cold, dry period from May to November. About 80% of the rainfall comes during the wet season, and the remaining 20% during the dry season in form of hail and snow (total rainfall of 300–900 mm). Weather data collected in the Department of Puno, where the La Raya High-Altitude Research Station is located, from 1931 to 1963 show the mean daily minimum temperature to be –3°C and the mean daily maximum temperature 17.3°C.[7] As in other high-altitude regions, the diurnal variation in temperature is great, at times exceeding 30°C.[8] This high-altitude environment is characterized by low atmospheric pressure, very dry climate, low oxygen availability, and intense solar radiation. In thin air, bodies absorb heat rapidly from the sun, and they lose it quickly when the rays of the sun are blocked by clouds. Moreover, wind, dry air, and low atmospheric pressure all are factors that increase the rate of evaporation.[7]

Because of the rugged climatic and geographic conditions, little or no agriculture is possible in this zone. Consequently, grazing of camelids and, to a lesser extent, sheep is the only means of subsistence for inhabitants of the highlands. With traditional herding practices, low-quality natural pastures can be converted into useful animal products.

History of International Exportations

The first contact of Europeans with South American camelids probably was that of Captain Francisco Pizarro's crew when he first arrived at the port of Tumbes in northern Perú, some time in 1528. Pizarro took some llamas back to Spain and displayed them at the Spaniard court. In those days, llamas were known by the Spaniards as *carneros de la tierra* ("rams of the land") and later as *carneros de cuello largo* ("long-necked rams").[3] Since that time, many attempts were made to introduce members of the South American camelid family to Spain, England, France, Germany, and Holland during the 16th, 17th, and early 18th centuries. Some accounts of these introductions are available at the literature.[9–11] By 1770 the government of Holland auctioned a herd of 32 alpacas and llamas belonging to King Wilfred II. It is said that this herd was purchased by the Frenchman Buffon, known in modern times as the "father of veterinary science," and sent to the Veterinary School of Alfort, where the present-day museum's exhibits include a beautiful dissection of a llama done by the anatomist Fragonard and hundreds of anatomic parts dissected by the best 18th century anatomists.

The greatest number of South American camelids ever carried to Europe at one time was a herd that arrived in Cadiz in 1808. It consisted of 36 animals, including llamas, alpacas, and vicuñas. They were brought from the highland of Peru to Buenos Aires, on the Atlantic Coast, by slowly travelling 2 to 3 leagues a day, and then shipped to Cadiz. Only 11 animals arrived at Cadiz alive, 2 of which died there. These animals were carried to Europe as a present from the Prince of Peace, Godoy, to Empress Josephine. In one report, six alpacas were imported to New Hampshire in the United States in 1849.[3]

One of the most interesting accounts of the introduction of alpacas and llamas to other countries is that of Charles Ledger, who wrote about his attempts to introduce them into Australia. He was particularly impressed by camelid adaptability: "No animal in the criation [sic], it is my firm conviction, is less affected by the changes of climate and food." In 1858, he landed 274 animals in Sydney after 4 months at sea. In 1864, however, the Australian government decided to abandon the alpaca business entirely, under the duress of the merino sheep breeders, who saw in the alpaca a fine wool producer and a future competitor.[12]

In the late 1800s, the llama was introduced into United States as a zoo exhibit; the number of imports was small and generally included guanacos or guanaco hybrids. One of the more significant importations was made in the early 1900s into the California coastal area by W. R. Hearst. In 1930, U.S. importation of camelids was cut off by a "foot and mouth disease" embargo on all South American hoofed stock. Finally, in the early 1980s, some American llama breeders started to import llamas and alpacas from Chile, and now the llama and the alpaca have developed from a zoo curiosity to the focus of a multimillion dollar industry growing at an exceptional rate. In the last 15 years, a total of 30,000 llamas and alpacas have been exported from Chile, Bolivia, and Peru to the United States, Canada, Spain, Israel, Ecuador, Libya, United Arab Emirates, Australia, New Zealand, Italy, Germany, and elsewhere.

Genetic Diversity, Distribution, and Number

Available census data for current populations of llamas and alpacas in the Andean Region are presented in Table 113-1.[13–17] The area of greatest concentration is located between 11 degrees south and 21 degrees south. Eighty-eight percent of the South American alpaca population are found in Peru. Likewise, the greatest population of llamas is found in Bolivia (62.5%). The rest of the alpaca and llama populations inhabit Chile and Argentina, and a very few are raised in Ecuador.

South American domestic camelids bred in different geographic zones of the Andean Mountains have sometimes been given local names, but it would be misleading to call them breeds. A survey carried out in 1991[13] studied the genetics of alpacas and llamas from Bolivia, Chile, and Peru, with the following findings:

Table **113-1**

Estimated Population and Distribution of Alpacas and Llamas in the Andean Region

Country	Alpacas	Llamas	Total	% Regional
Argentina	Few	75,000 (2%)	75,000	1
Bolivia	324,326 (11%)	2,022,126 (63%)	2,346,452	37
Chile	30,657 (1%)	66,383 (2%)	97,040	2
Peru	2,687,363	1,070,541	3,757,904	60
Total	3,042,346	3,234,050	6,276,394	100
Percent*	49	51	100	

*Percent of the total domestic camelid population.
Data from references 13 to 17.

Table **113-2**

Regional Names for Different Breeds of Alpaca and Llama

Country	Alpaca Breeds	Llama Breeds
Bolivia	Huacaya/Suri	Tampully/Kcara
Chile	Huacaya	Tajulli/Lutica
Peru	Huacaya/Suri	Chaku/Kara

Data from Sumar J: Data on alpacas and llamas: population and genetic diversity. EAAP/FAO information on livestock population. Data bank on animal genetic resources, Institute for Animal Breeding and Genetics, Hannover School of Veterinary Medicine, Hannover, Germany; and from Sumar J: Características de las poblaciones de llamas y alpacas en la sierra sur del Perú. In *Informe de la mesa redonda sobre camelidos Sudamericanos.* FAO/INIA, Lima, Peru, 1991, vol VI, pp 71–78.

Table **113-3**

Prevalence of Alpaca and Llama Breeds in the Andean Region

	ALPACA BREEDS		LLAMA BREEDS*	
Country	% Huacaya	% Suri	% Kara	% Chaku
Bolivia	100	—†	80	20
Chile	100	—†	60	40
Peru	99	1	80	20

*10% of the llamas are considered to be of an "intermediate type," the result of crosses between both breeds of llamas.
†There is a small number of specimens of the Suri breed.

1. Peru has two breeds of alpaca: the *Huacaya* (99% of the total alpaca population) and *Suri* (1% of the total alpaca population). Two breeds of llama are recognized: the *Kara* or nonwoolly species (accounting for 80% of the total llama population) and the *Chaku* or woolly species (which makes up 20% of the of the total llama population).
2. Bolivia and Chile have only one breed of alpaca, the *Huacaya,* representing 100% of the total alpaca population in those countries, although a few alpacas of the Suri breed live in the northern part of Bolivia, close to the Peruvian border. Of note in this context, since 1992, a number of the Peruvian breed *Suri* have been transferred to Chile for breeding. Likewise, Chile has two breeds of llama: the *Lutica* (60% of the total llama population), called *Kara or Kcara* in Peru and Bolivia, and the *Tampulli or Tajulli* (40% of the total llama population), called *Tampully* in Bolivia and *Chaku* in Peru (Tables 113-2 and 113-3).
3. It has been observed that approximately 20% of llamas in the three countries are of the "intermediate type"—that is, the result of crosses between animals of both breeds; also, crosses between alpacas and llamas also have been observed. In Peru, it is not uncommon to find intermediate phenotypes of alpaca (resulting from crosses between alpacas of the Huacaya and Suri breeds).
4. Tables 113-4 to 113-5 summarize data on coat color in alpacas and llamas of different breeds in Bolivia, Chile, and Peru.

CAMELID FARM CHARACTERISTICS

At present, 95% of all llamas and 75% of all alpacas in the Andean Region are under the control of traditional pastoralism. These alpacas and llamas have survived within the framework of a traditional, non-European, socioeconomic organization because they constitute an essential element of the Andean culture.[3] Typically, small herd holders maintain flocks of 30 to 300 head on communal grazing lands.[18] Most herders, however, include multiple species of domestic animals in their flocks, with alpacas and llamas being the dominant species. In the *puna,* mixed herds of alpacas, llamas, and sheep are quite common. Sometimes cattle graze along with the rest of the animals.

Table **113-4**

Coat Color Frequency in Alpacas

	% HUACAYA			% SURI		
Coat Color	**Bolivia**	**Chile**	**Peru**	**Bolivia**	**Chile**	**Peru**
Brown	41.0	52.0	23.5	—	—	32.0
Black	15.0	13.0	4.0	—	—	3.0
White	13.5	11.5	56.5	—	—	50.0
Gray	—	5.5	1.0	—	—	1.0
Roan	5.0	5.5	1.0	—	—	—
Multicolored	17.5	13.5	14.0	—	—	14.0
Total	100.0	100.0	100.0	—	—	100.0

Data from Sumar J: Data on alpacas and llamas: population and genetic diversity. EAAP/FAO information on livestock population. Data bank on animal genetic resources, Institute for Animal Breeding and Genetics, Hannover School of Veterinary Medicine, Hannover, Germany; and from Sumar J: Características de las poblaciones de llamas y alpacas en la sierra sur del Perú. In *Informe de la mesa redonda sobre camelidos Sudamericanos.* FAO/INIA, Lima, Peru, 1991, vol VI, pp 71–78.

Table **113-5**

Coat Color Frequency in Llamas

	FREQUENCY (%)		
Coat Color	**Bolivia**	**Chile**	**Peru**
Brown	23	11	25
Black	12.5	1.5	1.5
White*	11.0	12.0	33.0
Gray	9.5	6.0	9.5
Roan	5.0	5.5	—
Multicolored	43.0	69.0	31.0

*A very few animals were solid white.
Data from Sumar J: Data on alpacas and llamas: population and genetic diversity. EAAP/FAO information on livestock population. Data bank on animal genetic resources, Institute for Animal Breeding and Genetics, Hannover School of Veterinary Medicine, Hannover, Germany; and from Sumar J: Características de las poblaciones de llamas y alpacas en la sierra sur del Perú. In *Informe de la mesa redonda sobre camelidos Sudamericanos.* FAO/INIA, Lima, Peru, 1991, vol VI, pp 71–78.

Intensity of herd management varies greatly between producers, but in general, herders employ only traditional management. Herding practices typically are governed by availability of grass and community custom.[20] Little attention is given to protecting vegetation from overgrazing or to separating herds by specific species and classes. No attempt is made to provide supplementary feed for alpacas and llamas, resulting in low reproductive efficiency, high offspring mortality, parasitism, and consequently low animal productivity.

Regarding health, infectious diseases are important in this species. Neonatal enterotoxemia may kill as many as 70% of the crias.[21] Parasitic diseases are no less important as causes of reproductive inefficiency, particularly mange and parasitic gastroenteritis, which retard growth and decrease fiber yields and meat production.

Most alpaca and llama herds are maintained without any definite breed improvement criteria. Possible high levels of inbreeding due to small flock sizes also may potentially contribute to the low reproductive rate. Shearing is not done at regular intervals, typically being performed as a means to get money for a specific purpose.[3]

By contrast, better herd management practices are used on larger cooperative farms. In most of these large, mainly alpaca farms, animals are classified according to breed (Huacaya or Suri), coat color, age, and sex and maintained in groups of 200 to 800. The proportion of females of breeding age, however, usually varies, ranging from 34% to 57%,[18] whereas the proportion of castrated males kept as fiber producers may be 31% of the total farm total population. Shearing is mainly annual and in some cases biannual. From a practical standpoint, annual shearing is recommended because the fleece will be less damaged by the environment (e.g., solar radiation, rain, dust, dryness), allowing better external parasite control, and annual weighing of the live animal and its fleece allows for better control of production and genetic selection.

At present, culling of animals is based mainly on age: Mostly females older than 10 years of age and surplus males are sent to the slaughterhouse.

Reproduction and Survival of Offspring

Research done in the last 20 years has shown that biologic principles governing reproduction in Camelids are peculiar to the species and must be taken into account in applying techniques to improve birth rate and survival. For optimal breeding efficiency, females should not be bred until 10 to 15 days post partum, because earlier

matings can lead to uterine infections and would entail unnecessary use of stud animals.[23]

Neonatal mortality rates can be very high. In one investigation, the neonatal mortality rate for small herds was reported to be between 18% and 50%, and the rate for cooperative farms was higher, reaching 70% in some instances (Centro de Investigación IVITA, 1981). *Clostridium perfringens* type A enterotoxemia, which commonly occurs during the birthing season (very rainy season).[21]

Mating Systems

Alpacas and llamas are considered to be seasonal breeders. The breeding season occurs from the end of December to March (summer rainy months in the South Hemisphere). In small herds, in which males and females run together year-round, parturition also is restricted to the rainy months, as the gestation length is around 345 days.[24]

In better-managed herds, however, in which males and females are kept in separate herds, the females are bred using a specific male-to-female ratio; the males are introduced into the female herds at a rate of 6% for the entire breeding period of approximately 60 days.[25]

Half of the males are used for 1 week; then the other half are used also for 1 week, in an alternate system, to avoid the continuous association of males and females, which seems to inhibit libido in males. Use of this alternate or rotary breeding system on large alpaca farms in Peru resulted in an increase in birth rate from 57% to 81%.[25] This method of mating works well for mass selection, crossing the "best with the best," when it is not necessary to know the sire.

In those herds for which knowledge of sire identity is necessary (as required by the Peruvian Alpaca Registry), the system used is to join 20 females and 1 male in a fenced paddock for 60 days. A pregnancy rate of 70% is obtained with this method. The disadvantage of this method is the high cost of fencing large areas of pasture to keep the mating groups separate while providing the animals with enough food and water. Nevertheless, one of the advantages is that it allows testing of reproductive performance of the stud animal, with a required yearly minimum of 7 crias (offspring) from each male.

High rates of pregnancy have been obtained using **hand mating**, in which the females are checked daily for sexual receptivity and mated when appropriate with a proven fertile male.[26]

Peruvian Alpaca Registry

The Peruvian Alpaca Registry was established in 1995. The initial objectives of the registry were (1) to identify alpacas with certain fleece and conformation characteristics, allowing breeders or owners to make use of their best animals to improve offspring productivity, generation after generation; (2) to establish accurate records for pedigrees and other genetic studies; and (3) to avoid the exportation of the best alpacas—typically, those that win top prizes in the National Alpaca Show—in order to avoid what is called *genetic erosion*.

All alpacas graded higher than 75 points (100 points is the score for the ideal alpaca or the prototype) are recorded in the so-called **open book**, the first stage in the process of registry entry. In the second stage, only the offspring of those males and females recorded in the open book are registered. This is the initial step in establishing the pedigree. For the screening process, conducted under the auspices of the Consejo Nacional de Camelidos Sudamericanos (CONACS) (i.e., the National Council of South American Camelids), each alpaca presented to the examiner must score more than 75 points on the Alpaca Characteristics Evaluation Form. The Peruvian Registry assigns 30 points to body conformation and 70 points to fleece characteristics, and 40 points is given to fineness of the fleece. Alpacas must have a permanent double identification consisting of metal or plastic ear tags.

A **pedigree** is simply a record of ancestry, and most problems in practice arise over the value of such records.[27] The pedigreed animal has only an officially registered name and number assigned by the Livestock Association, which are of very limited value. For the pedigree to become a useful tool in alpaca production and improvement, official records for individual animals must include performance data. Originally, when pedigrees were written down by the first breeders, an animal's name was all that was necessary, because it was believed that everyone knew how it performed. This may still be the situation in the alpaca and llama industry in some countries, where the names of certain alpacas are internationally famous because they came from a certain alpaca farm, or because they have won many blue ribbons.

Before the establishment of the Alpaca Registry in Peru, a few breeders actually kept records of some kind that permitted assessment of the quality of the animal examined. In this manner, some breeders successfully improved their herds. The scientific basis for such records is very similar to what in modern genetics management is called a *performance test;* however, the accuracy of the data may not be highly reliable.

Because the fleece constitutes the main source of income for the Peruvian alpaca breeder, data on amount and quality of the fleece produced for some lines of alpacas are of considerable value. Reflecting the genetic diversity among Peruvian alpacas, fleece weights may range from 3 to 12 pounds in adult alpacas sheared annually. The management goal is intensive breeding of those alpacas with the highest fleece quality and weight.

Disease, Vaccination, and Deworming Practices

Infectious and parasitic diseases that affect alpacas and llamas play a prominent role in the economics of animal production owing to associated high mortality rates and decrease in productivity.

Both species are susceptible to most of the infectious diseases affecting other domestic animals. In mixed herds in the highlands of the Andean Region, cross-infections occur between the different animal species.[3] Camelids

are particularly sensitive, however, to some infections and relatively resistant to others. The introduction of camelids to other continents have resulted in the presentation of many new diseases not reported in South America. Whether or not alpacas and llamas are susceptible to a number of important viral diseases of cattle, sheep and horses is still unknown. Perhaps these animals have not been exposed to these viruses, or clinicians and researchers may not have conducted adequate virologic testing to discover them.[28]

Enterotoxemia is the most important infectious disease of neonatal domestic camelids. This disease is characterized by sudden death, with neonatal mortality rates as high as 70% during the first month of life. *Clostridium perfringens* types A and C are involved.[21] In adult alpacas, so-called alpaca fever, a bacterial septicemia caused by *Streptococcus zooepidemicus,* is associated with high morbidity and low mortality; this disease can be prevented by minimizing stressful situations. Other diseases in alpacas and llamas that have been diagnosed are osteomyelitis of the mandible, brucellosis, necrotic stomatitis, otitis, tetanus, tuberculosis, and keratoconjunctivitis. The prevalence of viral diseases—contagious ecthyma, rabies, herpes virus, adenovirus, bovine viral diarrhea, and foot-and-mouth disease (FMD)—is unknown. Infectivity and severity of FMD in alpacas and llamas, however, appear to be very much lower than in either cattle or sheep.[28] Herd health programs for domestic camelids in the Andean Region, do not include any kind of vaccination. Several vaccines have been advocated for use in alpacas and llamas, especially against enterotoxemia, although the efficacy of such vaccines and the optimal vaccination schedule remain unknown.

Both endoparasites and ectoparasites[29] cause considerable losses among camelids. Gastrointestinal parasites are reported to cause significant disease problems in the llama and the alpaca. Fecal examinations have recovered species-specific parasites including *Graphinema, Spiculoteragia, Camelostrongylus, Nematodirus lamae,* and *Lamanema*. In addition, *Ostertagia, Trichostrongylus, Cooperia, Nematodirus, Bunostomum, Oesophagostomum, Trichuris, Capillaria,* and *Haemonchus* are well recognized to occur. Many anthelmintics, although not specifically labeled for treatment of gastrointestinal parasitism in camelids, can be used safely. Fenbendazole and ivermectin provide the most effective and safest treatment, eradicating mature gastrointestinal parasites and effective against the dormant stages of these parasites. Liver fluke, *Distoma hepatica,* also occurs in domestic camelids, even at high altitude, although such environmental conditions are hostile to the intermediate host, snails. Coccidiosis due to species of the protozoan *Eimeria* also may affect young animals after weaning, causing diarrhea. Clinical coccidiosis is treated with sulfonamides and can be prevented by good management practices. Finally, sarcocystosis, caused by the coccidial protozoan *Sarcocystis,* can be prevented by deworming dogs, cats, and humans; prevention is important because there is no treatment for this disease. Alpacas and llamas are considered equally susceptible to these diseases.

Of the external parasites, the most common are the *Sarcoptes* and *Psoroptes* agents of mange; the scabies mite burrowing within the epidermis causes a papular dermatitis. The affected regions become alopecic, crusted, thickened, and hyperpigmented. Many therapies have been used for mange in camelids. Recently a popular agent for mange treatment has been ivermectin formulated for bovine use.

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CHAPTER 114

Reproductive Management of Llamas and Alpacas

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Llamas and alpacas have grown in popularity in the United States. These large domestic farm animals are commonly encountered by today's veterinary practitioners.

In 2002 the Alpaca Registry reported 42,000 alpacas owned by greater than 4000 owners in North America.[1] That report shows that concentrations of alpacas are greatest on the west coast, in the western Midwest, and on the northeast coast of the United States, with fewer alpacas in southern states. In Canada, the provinces of Alberta and British Columbia have the most registered alpacas. These numbers reflect only registered animals. The International Llama Registry (ILR)[2] reports greater than 5500 llamas in Canada and greater than 144,000 in the United States. The distribution of areas with the highest numbers of llamas is similar to that for alpacas in both countries. The ILR reported greater than 11,000 owners and greater than 350 owners of registered llamas in the United States and Canada, respectively. We believe that the reported number of registered animals is closer to the total number of animals, both registered and unregistered, for alpacas than for llamas. There are probably many more unregistered llamas than unregistered alpacas. Both alpaca and llama ranches appear to occur

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CHAPTER 114

Reproductive Management of Llamas and Alpacas

DAVID GARTRELL PUGH and MARY BETH STANTON

Llamas and alpacas have grown in popularity in the United States. These large domestic farm animals are commonly encountered by today's veterinary practitioners.

In 2002 the Alpaca Registry reported 42,000 alpacas owned by greater than 4000 owners in North America.[1] That report shows that concentrations of alpacas are greatest on the west coast, in the western Midwest, and on the northeast coast of the United States, with fewer alpacas in southern states. In Canada, the provinces of Alberta and British Columbia have the most registered alpacas. These numbers reflect only registered animals. The International Llama Registry (ILR)[2] reports greater than 5500 llamas in Canada and greater than 144,000 in the United States. The distribution of areas with the highest numbers of llamas is similar to that for alpacas in both countries. The ILR reported greater than 11,000 owners and greater than 350 owners of registered llamas in the United States and Canada, respectively. We believe that the reported number of registered animals is closer to the total number of animals, both registered and unregistered, for alpacas than for llamas. There are probably many more unregistered llamas than unregistered alpacas. Both alpaca and llama ranches appear to occur

in all sizes, ranging from very large (with greater than 1000 breeding females) to very small (with only 1 or 2 animals). In the authors' experience, alpacas tend to be handled, bred, fed, and managed in a more intensive manner than that used with llamas.

Llama and alpaca owners vary greatly in animal husbandry experience and in knowledge of these species. The clinician may be called on to be very involved with all aspects of farm management. The ownership of these species is expanding, and many new owners have little in the way of livestock experience. With some experience and acquaintance with the subtle differences between llama and alpaca breeding, feeding, and health maintenance, a practical understanding of small ruminant medicine will enable veterinary practitioners to easily include llamas and alpacas as a profitable and interesting part of their practice.

NUTRITIONAL MANAGEMENT

Llamas and alpacas have three compartments in the forestomach that are referred to simply as compartments C1, C2, and C3. C1 and C2 contain 85% to 90% of the total stomach volume and function as fermentation vats, similar to the rumen and reticulum of cattle, sheep, and goats.[3,4] The first compartment contains numerous saccules lined with glandular epithelium that aid in the absorption of water and solutes[3] and will retain fibrous feedstuffs longer (up to 60 hours) than in sheep (up to 40 hours).[5] The cranial portion of C3 also functions as a fermentation vat, whereas the caudal portion of the compartment is "abomasum-like."[4] Llamas and alpacas appear to have greater salivary production relative to foregut volume, and a faster liquid flow rate from the forestomachs, than are reported for sheep.[5] This faster liquid passage may result in quicker forestomach removal of soluble minerals, vitamins, and microbial protein.[5] Llamas and alpacas are capable of thriving on a diet that contains less dry matter on a body weight basis (1.2% to 1.8%) than has been recognized for many other herbaceous animals (greater than 2.5%). Llamas and alpacas may have a 10% to 25% greater efficiency in digestion and assimilation of nutrients than that in true ruminants, but when they are fed nutritionally good- to excellent-quality feedstuffs, this advantage appears to be lost.[3–6] Llama and alpaca nutrition has been discussed elsewhere, and interested readers should refer to those publications for further information.[3–6]

The llama is a browser by nature but will graze, whereas the alpaca is a grazer that shows a preference for succulent forages. Both species appear to be very adaptable to many types of feeding systems.[3,4] Cereal grains or pelleted supplements are rarely indicated except in older, chronically thin or ill animals, during prolonged periods of cold stress, or to ensure adequate intake of specific nutrients (e.g., selenium, zinc, protein).[4] The assessment of body condition helps in determining long-term nutritional intake, particularly with respect to energy. A 10-point scoring system is most commonly used in which 1 is very thin, 10 is extremely obese, and 5 is ideal.[3]

Body condition in llamas and alpacas is best assessed by palpating the transverse processes of the lumbar vertebrae, around the shoulders, and over the ribs. A convex or concave plane from the dorsal spine to the tip of the transverse processes would be considered to indicate condition scores greater than 5 and less than 5, respectively. If the ribs are easily palpated, the condition score is less than 5, but if the ribs are difficult to feel and if fat cover over the lumbar vertebrae is bulging and slightly soft, body condition for that animal is scored at 6 or greater.[4] The lateral aspects of the transverse processes of the lumbar vertebrae should not be sharp but should be easily palpable. The shoulder structures also should be palpable, with the bones and joint edges not sharp but appearing to have a slight smoothness. With weight gain, subcutaneous fat accumulates over the brisket, between the rear legs, and around the perineum. If the brisket is flat or soft between the front legs, then the body condition is 8. If the sternum is sharp and easily palpated, the condition score would be closer to 3. The pelvic bones can be easily palpated in all but obese llamas and alpacas.[4] When possible, body weights should be evaluated on a continuous basis, and steps should be taken to minimize body weight changes. Llamas and alpacas naturally gain weight in spring and early summer and tend to lose weight in late summer, fall, and winter. If they are weighed at 60-day intervals, adults that do not show this seasonal pattern, but continue to gain (or lose) weight, should be examined, and appropriate dietary changes made.[4]

Llamas and alpacas that are healthy, parasite-free, nonpregnant or nonlactating, and mature may have their nutritional requirements met by being fed 1.2% to 1.8% of their body weight daily of a diet that has an average of 55% total digestible nutrients, 8% to 10% crude protein, 25% crude fiber, 0.6% to 0.8% calcium, and 0.3% to 0.5% phosphorus.[4,5] These maintenance requirements usually are met by good-quality grass or grass-legume pastures or hay. All llamas and alpacas should have free access to fresh, clean water. Modification in energy intake may be needed during extremes in weather. In most instances, the mineral needs are met by offering a well-formulated trace mineral supplement as the only source of salt in a granulated form. Salt blocks do not permit adequate intake of trace minerals and are not recommended. In areas in which forages are badly deficient in phosphorus, selenium, zinc, or other minerals, diets may be designed to supplement such deficiencies. The clinician can aid in dietary management of the herd by nutritional analysis of pasture, hay, grain, and, when indicated, water. In cases of suspected mineral deficiency, adequacy can be assessed by tissue analysis (e.g., whole blood for selenium level, serum or plasma for copper and zinc levels).[4]

The requirements of adult males can be met by the recommendations previously discussed, with the exception that energy content be increased or decreased, depending on body condition. Male fertility can be depressed during periods of extreme high temperature and humidity.[4] To help minimize the incidence of heat stress, males should be shorn, fed highly digestible feeds (to avoid the increased heat of digestion associated with high-fiber feeds), fed to meet but not grossly exceed protein requirements, fed in the late afternoon or early morning, offered free-choice clean water, and maintained at a body condi-

tion score of 5 to 6. The hemostatic mechanisms used to dissipate heat (panting, sweating, and increased heart rate) may result in greater energy, mineral, and water requirements, and animals should be fed appropriately. Very aggressive males may lose excessive amounts of body weight and condition during a heavy breeding season.[7]

During the first two trimesters of gestation, dietary requirements will be met by feeding for maintenance. In the final trimester of gestation, mature females should be fed 1.5% to 1.8% of body weight of a good-quality forage, with a crude protein content of 10%. Energy requirements may increase to 1.5 times maintenance during the final trimester.[5] These requirements can be met by feeding a good-quality legume-grass pasture or hay with supplemental grain.[5] Overfeeding and obesity are common problems in llamas and alpacas in North America. Obesity should be avoided in breeding females because it may be associated with a higher incidence of heat stress, poor milk production, and dystocia.

During lactation, nutrient needs may increase by as much as 2 to 2.5 times maintenance requirements, particularly in heavy-milking females.[5] To meet these demands, total digestible nutrients and crude protein content of the ration should be increased to 60% to 65% and 12% to 15%, respectively,[4,5] Intake of some excellent milk-producing alpacas may approach as much as 2.2% to 2.5% of their body weight. These requirements can be met by good-quality legume-grass hay, fed on a free-choice basis, with some supplemental grain. The dam's diet can be reduced at the time her young are weaned (usually 3 to 5 months) to avoid obesity. Moderate energy restriction can be used for weight reduction in middle to late lactation for obese females; however, diets severely restricted in energy and protein should be avoided in late gestation because they may result in the formation of poor-quality or decreased quantities of colostrum, or in fatty liver syndrome.[4]

IMMUNIZATION AND PARASITE CONTROL

Vaccination guidelines for llamas and alpacas have been suggested, but the efficacy of these vaccine protocols has not been studied extensively in llamas and alpacas. Hence, vaccine use in camelids has been extrapolated from work performed in other species and from empirical use in field reports.[8–10] Bred females should be maintained on a program of yearly to biyearly immunization against *Clostridium perfringens* types C and D, *Clostridium tetani,* and perhaps other clostridial species *(Clostridium chauvoei, Clostridium novyii)* if disease due to these organisms is known to be endemic. In areas in which snakebite may be a problem, immunization for *Clostridium septicum* should be considered. Pregnant females can be vaccinated at 1 month before parturition to enhance colostral antibody content and to ensure adequate maternal immunity at time of parturition, particularly on ranches with a history of clostridial diseases of crias or where management is intense and crias are creep-fed.[4] Although many clinicians use seven-way or eight-way clostridial bacterins, these products are reported to be associated with abortions[8] and premature births.[4] We use three-way *Clostridium* bacterins unless specific diseases exist on a particular farm or ranch. If neonatal diarrhea is diagnosed, pregnant females may be immunized in late gestation with a commercially available vaccine for use in cattle. The use of these vaccines is off-label and not without risk, but this approach may be useful on some farms.

Although commercially prepared *Leptospira* vaccines are commonly used, the immunologic response to such vaccines in llamas and alpacas has been questioned.[11,12] If leptospirosis is endemic, strategic vaccinations every 3 to 4 months with multivalent vaccines should be strongly considered.[4] The clinician should resist a false sense of security when vaccinating against leptospirosis and should attempt to institute management practices that are effective in decreasing exposure. In areas in which llamas and alpacas have exposure to exotic equids (e.g., zebras), the use of a killed form of equine herpesvirus type 1 (EHV1) has been advocated.[9] These vaccines also may be useful in preventing bovine herpes in camelids. Two initial immunizations should be given, followed by boosters every 2 years. In areas endemic for rabies, the use of a killed product approved for use in ruminant species may provide protection. We recommend an initial vaccination at 4 to 6 months of age with a second immunization 2 weeks to a month later, followed by a yearly booster.[13] Bovine viral diarrhea (BVD) has been documented in both llamas and alpacas, but the prevalence is low.[14] Serologic testing of llamas and alpacas showed that exposure was less than 0.9% in the southeastern United States.[14] In areas or on farms where BVD has been documented as a problem, killed vaccine may provide a titer response and may be useful in BVD prophylaxis.[15]

Methods of parasite control are varied and, like vaccination programs, should be designed to logically fit the specific farm or ranch. Little in the way of basic research dealing with anthelmintic use in llamas or alpacas has been published to date, and dosages must be extrapolated from use in other species. The basic principles of parasite control are proper pasture management and rotation, adequate nutritional intake, and appropriate use of anthelmintics. When anthelmintics are decided on, dosage based on accurate body weight and proper administration route are imperative. As a general rule, only oral or injectable anthelmintics should be employed in llamas or alpacas. We authors can find no documented evidence that supports the idea that poor reproductive performance (e.g., abortion, infertility) or birth defects are linked to the use of any anthelmintic in llamas and alpacas. Anthelmintics that are associated with abortion or anomalous fetal development in other species (e.g., albendazole given during early gestation) should be avoided, or the prescribed precautions should be followed when such agents are used in llamas or alpacas.

BREEDING MANAGEMENT

In our experience, the most common cause of subfertility in llamas and alpacas is poor management. Breeding programs being used in North America are varied, and the clinician should be able to help design and implement a reproductive management system best suited for the

particular farm. The physical facilities, available labor, husbandry experience, and the number and type of animals are some of the parameters that aid in the decision regarding what type of breeding program to utilize. All breeding programs require complete, easily understood records. Accurate identification of animals is paramount in all breeding systems; therefore, each animal should be labeled, tagged, tattooed, or implanted with a unique, easily read identification number.[4]

A thorough understanding of the reproductive pattern of llamas and alpacas and the use of transrectal ultrasonography will enhance the clinician's ability to assist in reproductive management. The breeding and birthing seasons should be planned to minimize birth of crias during periods of extreme heat, humidity, or cold.[4] Females should be bred after 12 months of age and not before they attain 65% of projected mature adult weight. If animals have a body condition score greater than 6, then the female should have attained greater than 65% of the projected adult weight before breeding. Before the breeding season, all animals should be condition-scored, vaccinated, and dewormed and undergo all other health care practices (e.g., toenail trimming).[4]

Pasture breeding (in which one or more males are placed with a group of females) is commonplace in South America and in many large operations in North America. In some Peruvian pasture breeding programs, males are maintained at only 3% of the herd (3 males per 100 females). Up to 18 copulations per day are possible by males, particularly on the first day of presentation to females.[4,7,16] Close observation for breeding, accurate record keeping, and periodic (biweekly or monthly) reproductive examinations of females will help maximize effectiveness of pasture breeding.

In a modified form of pasture breeding, the males are removed after 7 to 14 days and reintroduced 14 to 21 days later. Females that refuse mating after reintroduction are examined for pregnancy (e.g., by transrectal ultrasonography). Such modified pasture breeding programs allow more accurate record keeping and more rapid identification of problem animals.[4]

Hand mating requires the most intense management and, if carried out properly, yields very high reproductive performance. As intensity of intervention in breeding increases, management of animals also must be dramatically improved. Inaccurate record keeping and poor or "sloppy" management practices will result in poor performance. An advantage of hand breeding is early identification of females with reproductive problems.

Males may be presented in hand or set free in a paddock with an individual female. Females with a mature ovarian follicle (greater than 7 mm in diameter) usually will assume sternal recumbency quickly when presented with the male. Considerable individual variation is possible, however, because some females are very protective of their cria or are very aggressive and may not show receptivity as readily. This procedure may be repeated, at frequencies ranging from daily to weekly, until the female refuses the male's advances. Successful hand breeding requires the ability to identify females at the most receptive stage of their cycle.[4] Good teasing protocols and records aid in identifying the best time to breed. Ultrasonographic examination of the reproductive tract enhances success of a hand breeding program, particularly if the findings are correlated with teasing.[4] Teasing should commence 10 to 14 days after breeding and continue on alternate days for 9 to 10 days. Females that continue to breed over three breeding cycles should be examined for abnormalities.

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CHAPTER 115

Reproductive Anatomy and Life Cycle of the Male and Female Llama and Alpaca

JULIO SUMAR and GREGG P. ADAMS

Present-day alpacas and llamas developed from a common feral ancestry in South America during the last millennium. Many aspects of their reproductive pattern are similar but extrapolation from one species to another must be done with caution. Although differences in the reproductive biology have not been documented, few critical studies have been conducted to compare the various species of camelids. Breeding experiences and research with llamas and alpacas under different latitudes and lower altitudes such as those being carried out in Australia, Canada, and the United States may soon contribute to a better understanding of the biology of reproduction in llamas and alpacas.

GROSS AND ULTRASONOGRAPHIC ANATOMY OF THE FEMALE

The gross appearance of the female reproductive organs in the alpaca is illustrated in Figures 115-1 and 115-2. The dimensions of alpaca and llama reproductive tracts are compared in Table 115-1.[1–3] The ultrasound appearance of the ovary is illustrated in Figure 115-3.[4] The ovum itself is too small to be detected by present-day ultrasound instruments, but antral follicles as small as 1 to 2 mm in diameter can be detected. Several fluid-filled follicles (e.g., from four to eight), with or without a corpus luteum, may be detected in the ovaries at any given time. On ultrasound examination, the appearance of the llama or alpaca ovary is more similar to that of the cow than of the mare. The ovary and ovarian structures are, however, smaller than in cows (e.g., ovulatory-sized follicle, 10 mm versus 16 mm in diameter, respectively; mature corpus luteum, 13 mm versus 28 mm, respectively). As in the cow but not the mare, ovarian follicles are arranged in a peripheral cortex, and ovulation can take place at any spot on the surface of the ovary. The corpus luteum and large follicles protrude distinctly from the surface of the ovary in llamas and are readily palpable. On ultrasonography, corpora lutea are seen to have a very characteristic spherical shape and are hypoechogenic (dark gray) relative to the surrounding tissues. A horizontal echogenic (light gray) area traversing the center of the corpus luteum also is a distinctive ultrasonographic feature. The capability to ultrasonographically recognize and monitor the development of the corpus luteum is fortuitous because it allows confirmation of ovulation and is a rapid method of "progesterone testing."

The oviducts are long and tortuous and end in an open bursa that normally covers the ovary. The tip of the uterine horns in both domestic species is blunt and

Table **115-1**

Dimensions of the Reproductive Organs of Female Llamas and Alpacas

	VALUE (cm): MEAN ± SD	
Organ Dimension	**Alpaca**	**Llama**
Length of labia	2.5	5.0
Vagina (hymen to cervix)		
Length	13.4 ± 2.0	15–21
Diameter	3.4 ± 0.7	5
Cervix		
Length	2	2–5
Diameter	—	2–4
Number of rings	2–3	2–3
Uterine body		
Length	3.0 ± 0.7	3–5
Diameter	—	3–5
Uterine horns		
Length	7.9 ± 1.3	8.5–15
Diameter	—	2.5–4
Oviduct length	20.4 ± 4.2	10.5–18.3
Right ovary		
Length	1.6 ± 0.3	1.3–2.5
Depth	1.1 ± 0.2	1.4–2.0
Width	1.1 ± 0.2	0.6–1.0
Left ovary		
Length	1.6 ± 0.3	1.5–2.5
Depth	1.1 ± 0.2	1.5–2.5
Width	1.1 ± 0.2	0.5–1.0

SD, standard deviation.

Data from Sato A, Montoya L: Aparato reproductor de la alpaca *(Lama pacos)*. Anatomía macroscópica. IVITA/CICCS, Universidad Nacional Mayor de San Marcos. *Revista de Camélidos Sudamericanos* 1990;7:23; Bravo PW, Sumar J: Some anatomical parameters of the reproductive tract in alpacas [in Spanish]. Resúmenes de inves, Universidad Nacional Mayor de San Marcos, Lima, Peru; Fowler ME: *Medicine and surgery of South American camelids: llama, alpaca, vicuña, guanaco.* Ames, IA: Iowa University Press, 1989; and Sumar J: Studies on reproductive pathology in alpacas. Master's thesis, Department of Obstetrics and Gynaecology, Veterinary Medicine Faculty, Swedish University of Agrarian Sciences, Uppsala, Sweden, 90 pp.

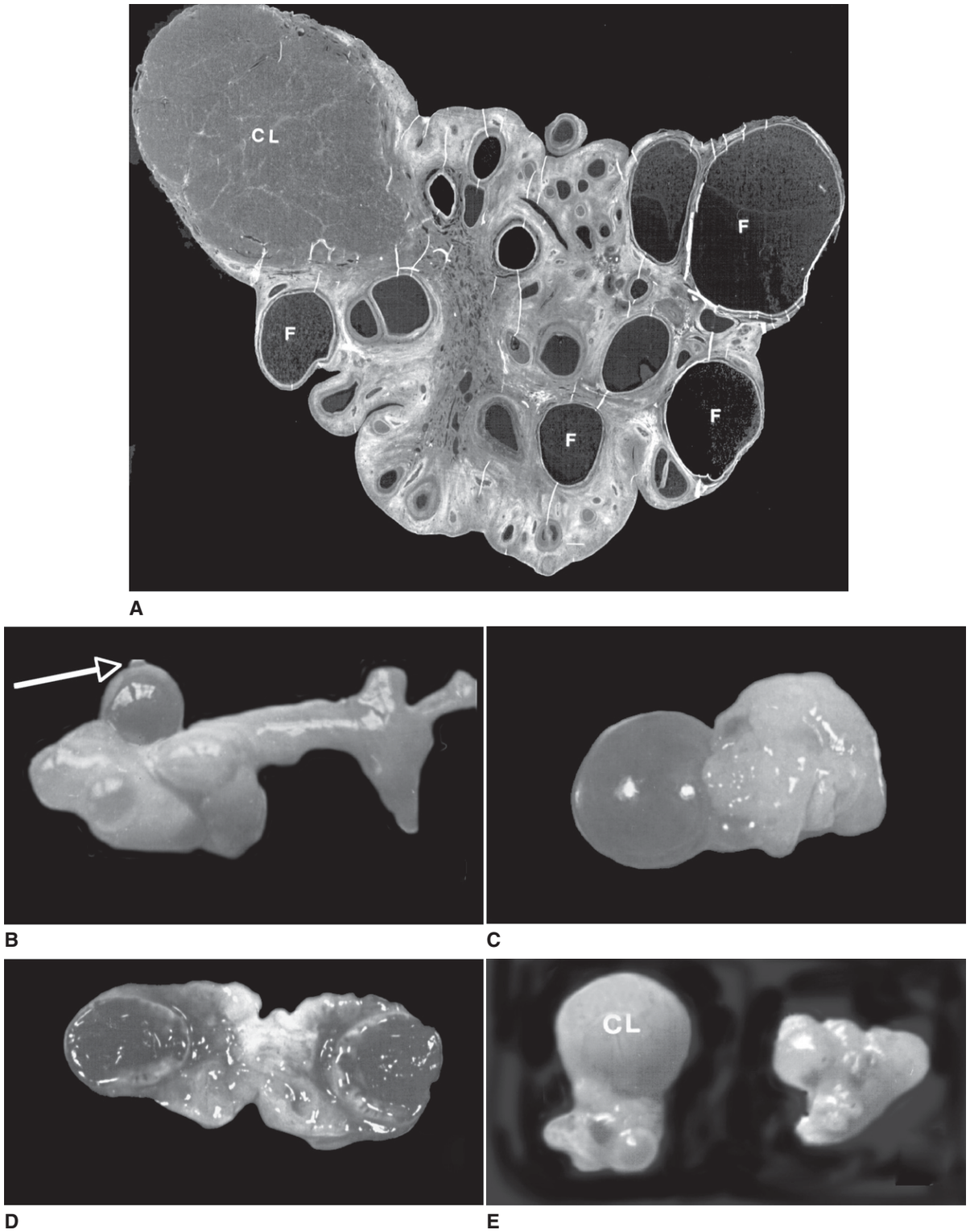


Fig. 115-1 Gross appearance of alpaca ovaries. **A,** Cross section showing a corpus luteum (CL) and numerous follicles (F). **B,** Recently ovulated follicle with an ovulatory papilla *(arrow).* **C,** Mature 12-mm follicle. **D,** Cross section of a corpus hemorrhagicum. **E,** Corpus luteum and several follicles in the ovaries of a pregnant alpaca (at 2 months of gestation).

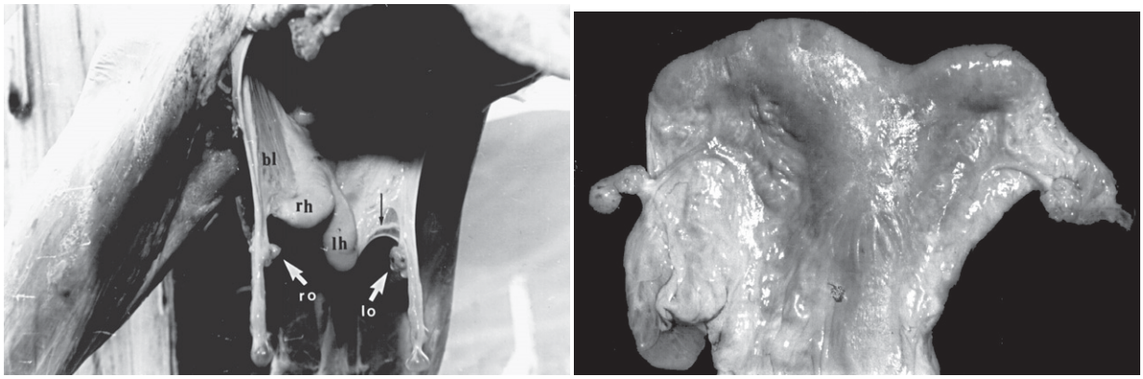


Fig. 115-2 Reproductive organs of the alpaca and llama. **A,** Craniolateral view of the suspended reproductive tract of an alpaca at slaughter showing the left and right uterine horns (lh, rh) suspended by the broad ligament (bl), the left and right ovaries (lo, ro), and the left oviduct *(black arrow).* **B,** Dorsal view of the excised tract of a llama. The uterine horns are relatively short, not tapered, and come to a blunt uterotubal junction, unlike in cows or ewes, in which the uterine horns are long and taper toward the uterotubal junction.

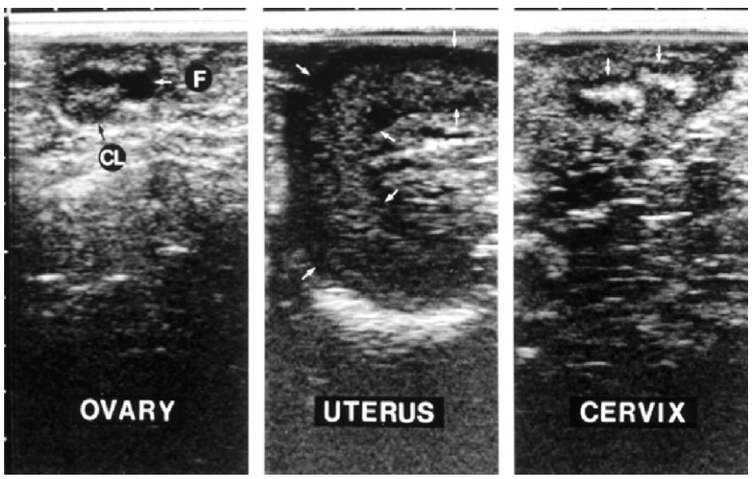


Fig. 115-3 Ultrasound images of the llama ovary, uterus, and cervix. Morphologic features are characteristic of the luteal phase:, a follicle and a corpus luteum in the ovary, curled uterine horn, and distinct cervical folds. The scale at the *top* is in 1-cm increments, and the images are in a sagittal plane with the cranial direction to the left.[4]

rounded, unlike in other ruminants, in which it tapers slowly toward the uterotubal junction. Accordingly, the oviduct of alpacas and llamas opens into the uterine horns through a small, raised papilla, which acts as a well-defined sphincter. Even under great pressure it is not possible to flush liquids from the uterus into the oviduct, but flushing in the opposite direction is possible. As in other domestic species, the uterus of the alpaca and llama is bicornuate, but the left uterine horn is slightly longer than the right (7.9 ± 1.3 cm versus 7.4 ± 0.9 cm), even in the nullipara.

In situ, the uterine horns curl ventrally and caudally, and the degree of curl is maximal during the luteal phase (progesterone dominance) and minimal during the follicular phase (estrogen dominance)[4] (Fig. 115-4). The degree of uterine horn curl is much less than in other ruminants (e.g., sheep, goats, cattle), and in the excised tract, the uterine horns shrink and may not appear curled at all, but rather appear Y-shaped. The echotexture (grain of the ultrasound image) of the uterus changes from homogeneous light gray (smooth and uniform) during the luteal phase and early pregnancy to heterogeneous and increasingly dark during the follicular phase (see Fig. 115-4). Estrogen causes the reproductive tissues to imbibe water and, since fluid is nonechogenic, the uterus becomes relatively hypoechogenic during the follicular phase. A similar pattern has been reported in cows.[5] In nonpregnant llamas, the body of the uterus is short (2 to 3 cm) and not clearly differentiated from the base of either horn on ultrasonography. Uterine tone, based on digital palpation, is maximal during the follicular phase and minimal during the luteal phase and early pregnancy, as in cattle. The uterus is remarkably turgid during the follicular phase, and longitudinal grooves can be palpated along its upper surface. Thus, both the homogeneous dark echotexture and extreme turgidity of the uterus indicate a profound accumulation of interstitial fluid during follicular dominance. The tubular genitalia are suspended

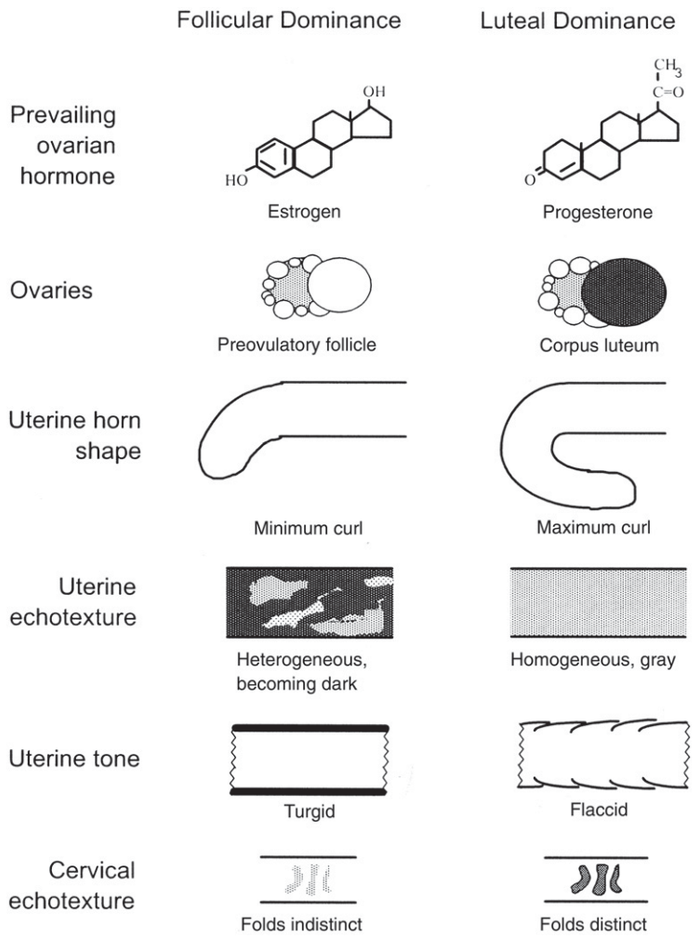


Fig. 115-4 Characteristic differences in the reproductive organs of alpacas and llamas during the follicular phase (receptive) versus the luteal phase (nonreceptive).[4]

from the lateral pelvic and abdominal walls by the broad ligament; the ligament attaches along the ventral aspect of the uterus such that the uterine body and horns are readily palpable per rectum.

The cervix has two or three irregular annular or spiral folds. On ultrasonography, the cervical folds appear as transverse echogenic bands and are especially prominent during the luteal phase and pregnancy (see Fig. 115-4). Presumably, edema of the cervical folds during the follicular phase is responsible for the dark echotexture and indistinct appearance on the sonogram characteristic of this phase. The vagina is surprisingly long and commonly exceeds 20 cm.

ANATOMY OF THE MALE

In the adult alpaca, the testes are found in a nonpendulous scrotum in the perineal region 5 to 9 cm ventral to the anus.[1,6] The testes are small and elliptical and positioned so that the long axis is oriented vertically or in an oblique dorsocaudal orientation. Normally, both testes are of the same size and of a firm consistency, with free movement inside the scrotum. The head, body, and tail of the epididymis are small and firmly connected to the testes. The average weight of one fully developed alpaca testis is approximately 17 g (range, 13 to 28 g), measuring between 4 and 5 cm in length and 2.5 to 3.0 cm in depth.[7] Considerable variation, however, has been found in testicle size and liveweight in alpacas. In one study, male llamas that reached the age of 2 years (83 kg of body weight) had testes weighing about 12 g, with dimensions of 3.5 × 2.2 cm and a volume of 13 cc. At the age of 5 years, llama testes weighed about 24 g, with dimensions of 5.0 cm × 2.7 cm and a volume of 22 cc.[9] In another study,[10] body weight and testicular index increased linearly from birth to 24 months of age, with a subsequent slower increase to adulthood. A positive relationship was found between age and testicular index ($R^2 = 0.88$).

The deferent duct (ductus deferens) is very thin (2 mm) at its beginning and thickens (3 mm) when it reaches the abdominal cavity and ends near the bladder, forming what in other species is the ampullae.[14] The prostate consists of a clearly defined body at the neck of the bladder, and a disseminate part along the pelvic urethra. The prostate body measures approximately 3 cm × 2 cm and is easily palpable per rectum in the adult. The bulbourethral glands are oval and are located on either side of the urethra at the pelvic outlet. Camelids have no vesicular glands.

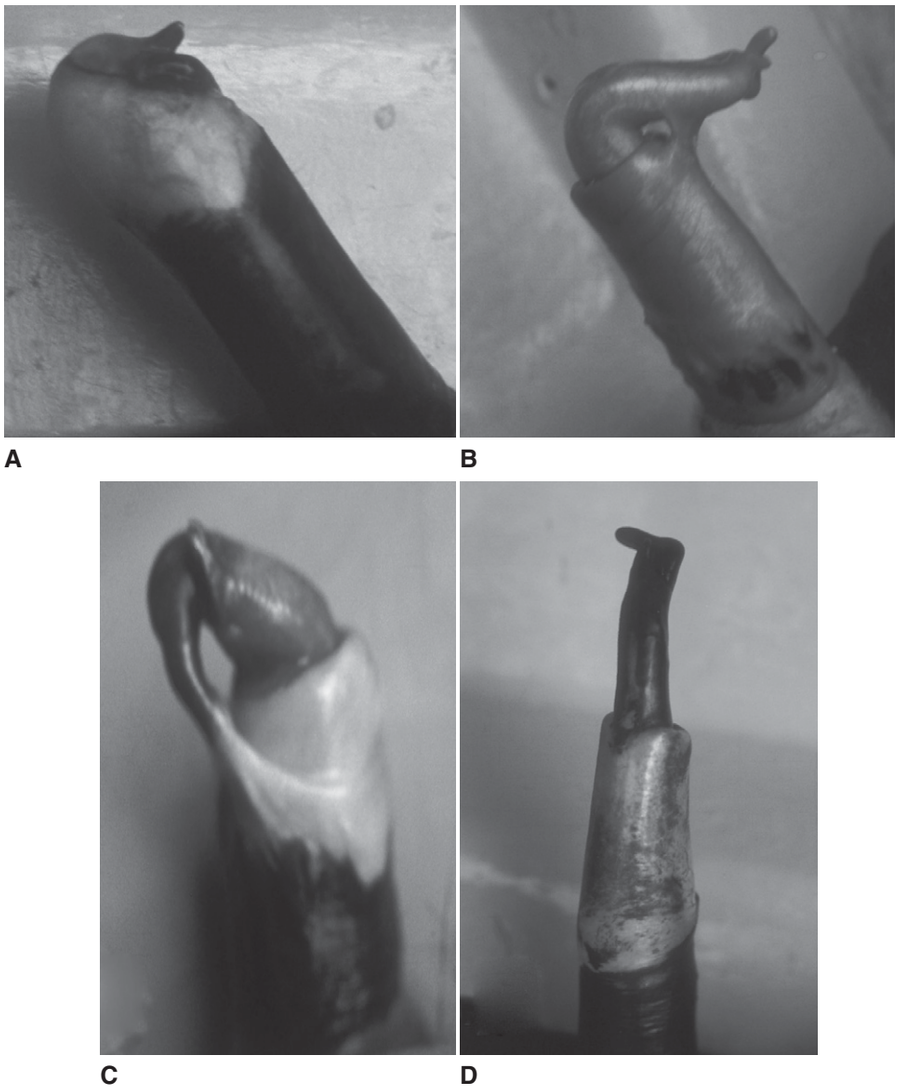


Fig. 115-5 Penis of alpacas showing progressive stages of separation of the free end of the penis from its preputial adhesions. **A,** Complete adhesion with only the tip of the cartilaginous process showing; **B,** the beginning of separation; **C,** persistent adhesion of the tip of the penis, and **D,** a penis completely free of its preputial adhesions.

The penis is fibroelastic, with a small (approximately 1 cm across) cartilaginous process that has a hooklike curve at its tip (Fig. 115-5). The length of the nonerect penis in the alpaca is 26 cm, and the sigmoid flexure is prescrotal.[6] The prepuce is small, located approximately 15 cm caudal to the umbilicus, and has a triangular opening that is oriented caudally.[1] During urination, camelids direct the stream of urine backward between the hind legs. The protractor preputial muscle pulls the prepuce forward before mating, changing the direction of the preputial opening and thus allowing for the penis to be directed forward.

PUBERTY

Young female alpacas of 12 to 13 months of age display behavioral estrus similar to that in adult alpacas.[11] A majority of females display sexual receptivity at 12 months of age even though ovarian activity begins at 10 months of age, with the presence of follicles of 5 mm or more in diameter. In a study carried out in southern Peru using 280 yearling alpaca females,[12] a highly significant relationship ($P < 0.001$) was found between body weight at mating and subsequent birth rates. For each kilogram increase in body weight up to 33 kg, a 5% increase in natality was observed; for increases in excess of 33 kg, the percentage of nonpregnant females was relatively independent of body weight. In traditional Peruvian production systems, only about 50% of yearling alpacas reach a body weight of 33 kg at mating time (January through March). Therefore, breeding age is postponed to 2 years of age and often to 3 years of age in llamas. It also has been shown that with better nutrition after weaning (7 to 8 months of age), nearly 100% of yearling alpacas can reach 33 kg body weight.[13]

In males, the penis is completely adherent to the prepuce at birth. Under the influence of testosterone, the adhesions disappear gradually as the male matures.[6,7]

At 1 year of age the males show sexual interest in the females, but only about 8% of alpaca males have complete liberation of penis-prepuce adhesions and are capable of copulating. At 2 years of age, approximately 70% of the males are free of adhesions, and 100% are free at 3 years of age (see Fig. 115-5). Puberty, based on penis-prepuce detachment, is attained at an average of 21 months of age (range, 9 to 31 months) in llamas, at a body weight of 70 kg (range, 48 to 92 kg).[10] Also, testosterone concentration starts to increase exponentially at 21 months of age (to 300 pg/mL), reaching a plateau at 30 months of age (650 pg/ml).[9,10] From 2 to 11 months, the testes grow slowly (infantile stage), with a sequential increase of noncanalized sex cords and undifferentiated Leydig cells.[14] At an age of about 12 months, the sex cords start to develop a distinct lumen containing Sertoli cells and spermatogonia. The first spermatozoa appear at about 18 months of age, with half of the animals having spermatozoa in the caput epididymidis. From 18 months, the number of Leydig cells increases considerably, and by 20 to 24 months, the diameter of the tubules is greatly increased, with presence of considerable numbers of spermatozoa in the caput epididymidis. Beginning at 3 years of age, normal alpacas have spermatozoa in the epididymis. Spermatogenesis is initiated in some alpacas as early as 16 months and in others as late as 26 months.[59] The general practice in Peru is to start using the males for breeding at 3 years of age.

SEASONALITY

Studies with alpacas and llamas in their natural habitat in the highlands of Peru showed that sexual activity is seasonal and lasts from December to March (summer months). These are the warmest months of the year, with sufficient rain and abundant green forage.[15] In farms on which males and females are together all year, the birthing and breeding times lie within this time range. This marked seasonality of reproduction also is characteristic of the wild species—the vicuña and guanaco.[16] Observations in different zoological parks of the world, however, indicate that llamas are year-round breeders.[17] In North America, where llamas are kept under good nutritional conditions year-round and copulation is allowed only on an individual basis, they are considered nonseasonal breeders. An analysis of llama birthing records from the Rocky Mountains area of the United States revealed that births occurred during all months of the year, but with a preponderance (73%) between June and December.[18] In another study, ovulation, fertilization, and embryo survival rates were not affected by the season of the year.[19]

Males show much less seasonal variation in libido and testicular function. The male alpaca is capable of producing fertile ejaculates year-round, but as in other domestic species, semen quality and libido are affected by season and availability of feed. Under North American conditions of management, no seasonal effect on testosterone values of adult male llamas has been found.[10] In a study of male alpacas and llamas in Peru, plasma testosterone concentrations were markedly elevated during spring and summer (the breeding season), whereas lower levels occurred in autumn and winter[20] (Table 115-2).

Table **115-2**

Seasonal Variation in Peripheral Testosterone Level in Male Alpacas and Llamas

	TESTOSTERONE CONCENTRATION (pg/ml): MEAN ± SEM	
Season: Month	**Alpacas**	**Llamas**
Spring: March	1142 ± 108	208 ± 52
Summer: June	992 ± 388	37 ± 14.9
Autumn: September	877 ± 91	291 ± 74
Winter: December	2445 ± 694	362 ± 73

SEM, standard error of the mean.
Data from Lichtenwalner AB, Woods GL, Weber JA: Male llama choice between receptive and nonreceptive females. *Appl Anim Behav Sci* 1998;59:349–356.

PATTERN OF OVARIAN EVENTS

Because copulation is a necessary prelude to ovulation, alpacas and llamas have been classified as **reflex ovulators** or **induced ovulators**, as opposed to spontaneous ovulators.[21,22] Female alpacas and llamas do not have estrous cycles like those in other species of large domestic animal; estrus and ovulation are not manifested in a repetitive cycle. Highlights of ovarian function are presented here, but a more detailed account is found in Chapter 118.

Based on laparoscopic examination of the ovaries of alpacas ($n = 20$)[23] and llamas ($n = 12$),[24] it was found that growth, maintenance, and regression of a follicle each required an average of 4 days (total, 12 days; range, 9 to 17 days). In a study of 74 llamas kept in their natural habitat in South America and examined daily by transrectal ultrasonography,[25] successive dominant follicles emerged at intervals of 19.8 ± 0.7 days in unmated and vasectomy-mated llamas and 14.8 ± 0.6 days in pregnant llamas.

The minimum time to ovulation was estimated to be 26 hours after natural mating and 24 hours after treatment with intramuscular human chorionic gonadotropin (hCG) at a dose of 500 to 700 IU.[21] Using an ultrasonographic technique in llamas, ovulation was detected, on average, 2 days (range, 1 to 3 days) after a single mating.[4,25] Single service by an intact or vasectomized male resulted in an ovulation rate of 77% to 82%, and an increase in the number of services by intact males to three within a period of 24 hours did not significantly affect ovulation rate.[15] In females allowed a single mating, 50% ovulated between 26 and 30 hours, 24% ovulated between 30 and 72 hours, and 26% failed to ovulate after mating.[26] Forty percent of the animals that failed to ovulate were yearlings, and 15% were adults.

Some evidence indicates that females can ovulate without coital stimulation or exogenous hormones, especially when initially isolated from and then reintroduced

to a male. The rate of spontaneous ovulation has been reported to be approximately 5% in alpacas.[6,15] Spontaneous ovulation was detected in 3 of 20 (15%) llamas by palpation in one study,[27] and in 3 of 34 (9%) llamas by ultrasonography in two other studies.[4,25]

Laterality of ovulation (from either the left or the right ovary), as indicated by the presence of a corpus luteum of pregnancy, has been reported in several studies.[6,24,26,28,29] On examinations of the genital organs of 928 pregnant alpacas, no differences were found in the ovulatory rate between ovaries. A corpus luteum was detected in the right ovary in 51% of the alpacas, in the left in 47%, and in both ovaries in 2%.[28] In the llama (n = 110), a corpus luteum was detected in the right ovary in 54% of females, in the left ovary in 44%, and in both ovaries in 1%.[29]

Luteal function after sterile and fertile matings has been studied in llamas by several investigators.[6,25,27,28,30] In nonpregnant alpacas, the corpus luteum underwent rapid development after ovulation, reached maximum size and secretory activity at 8 to 9 days after mating, and declined sharply in both size and secretory activity by 12 days after mating.[31] Similar characteristics were observed in nonpregnant llamas (i.e., mated to vasectomized males) evaluated by ultrasonography.[32] Although prolonged luteal phases have been anecdotally reported in association with embryonic loss, spontaneous pseudopregnancy (prolonged luteal phase unassociated with embryonic loss) has been wrongly inferred in llamas.[21] A prolonged luteal phase was not observed in any sterile-mated (nonpregnant) alpacas or llamas.[25,27,31,32] In pregnant animals, the corpus luteum reaches maximal diameter at 22 days after mating and is maintained throughout gestation.[32,33]

SEXUAL RECEPTIVITY AND MATING BEHAVIOR

In the absence of copulatory stimulation, female alpacas display periods of sexual receptivity of up to 36 days, with short periods of nonreceptivity that may last 48 hours.[21] Considerable variability in display of overt receptivity is apparent between individual animals, regardless of parity. The variability in sexual receptivity may be attributable primarily to the degree of follicle maturity at any given stage of the follicular phase; however, no documentation is available in this regard.

The receptive female will assume the prone position (ventral recumbency) after a short period of pursuit by the male, or she may approach a male that is copulating with another female and adopt the prone position[21] (Fig. 115-6). Some receptive females may occasionally display mounting behavior with other females of the herd, although such behavior is much less common than in cattle. If the female is nonreceptive, rejection is shown by running away and spitting at the male. Males will attempt to mount receptive or nonreceptive females; their initial approach is indiscriminate. In a study of sexual behavior in llamas, the male attempted to mount eight of nine females a total of 60 times during a 12-hour period.[20] Each of the eight females was mounted an average of four times, for an average of 5 minutes per mount; mounting was attempted irrespective of the receptivity of the female. Normally, only receptive females will adopt the prone position, but in some instances, aggressive males can force nonreceptive females to adopt the prone position. During the very short courting phase and during mating, the male makes blowing, grunting, nasal sounds. Copulation takes place in a recumbent position, with the male mounted above and just behind the female. The female assumes a very passive attitude during copulation and, in some instances when copulation is prolonged, will appear to tire and may assume lateral recumbency. Compared with other domestic species, coitus is remarkably prolonged in alpacas and llamas (10 to 50 minutes).[6] After a certain amount of time has elapsed, the continuous association of males and females somehow inhibits the sexual activity of the males, a situation that may prevent mating of females returning to estrus after a sterile mating or early embryonic death.

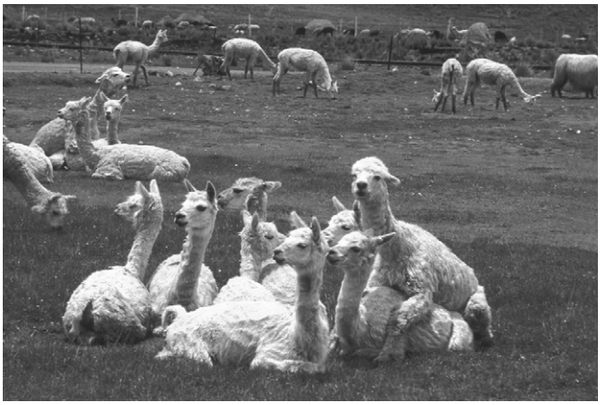
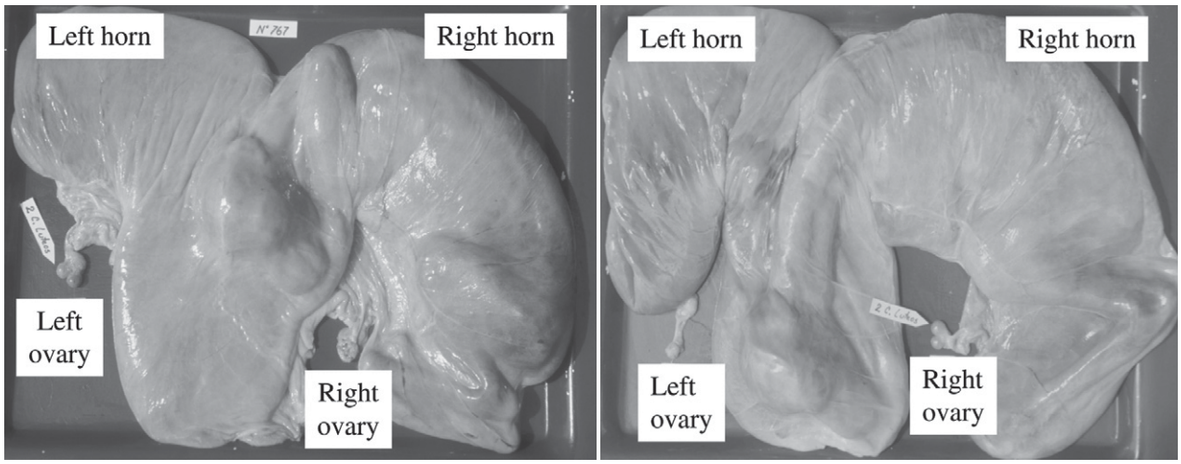


Fig. 115-6 A pair of copulating alpacas in a prone position, surrounded by six females also in the prone position, typical of a display of sexual receptivity.

MATING SYSTEMS

Observation of the sexual activity in the open field, when males and females are joined, at the beginning of the sexual season, showed that mating activity was particularly intense during the first week of the breeding season.[34] Greater than 70% of 262 females were recorded as having been mated at least once during this short period. But sexual activity decreased thereafter, reaching a frequency of zero in some instances. After different males were introduced and the original males were removed, however, sexual activity was reactivated and reached a level comparable to that of the first week.[35]

Based on these observations, a breeding system named the **alternating** or **rotary system** was developed.[36] This system works well in the large herds commonly found in southern Peru. Males are used at a stocking rate of 6% for the entire breeding period of 60 days. Half of the males are used for 1 week and are then rotated the following week with the other half. By alternating the males in this manner, libido and mating activity remain high, and the opportunity for the females to be mated once or more may be maximized. Use of the rotary system in a large alpaca cooperative in Peru (adults and yearlings, n = 1399) resulted in a birth rate increase from 57% to 81%.[36]



A B

Fig. 115-7 Unusual right-sided pregnancy in alpacas (approximately 8 to 9 months) with the corpus luteum *(arrow)* in the left *(left image)* or right *(right image)* ovary.

PREGNANCY

The duration of gestation in the alpacas of the Huacaya and Suri breeds has been reported as 341 and 345 days, respectively.[21] For llamas in the United States, a mean of 344 days (range, 331 to 347) was reported.[18] In one study of 79 nulliparous and 61 multiparous llamas in southern Peru in which only two matings were allowed (at an interval of 6 to 8 hours apart), the length of the gestational period (mean ± SD) was 346 ± 8 days (range, 327–357).[37] Neither parity of the dam nor sex of the cria was found to influence gestation length.

Despite the fact that ovulation occurs from both ovaries with equal frequency, nearly all alpaca and llama fetuses occupy the left uterine horn (as indicated by position of the conceptus and site of umbilical attachment), with rare exceptions (Fig. 115-7). This finding indicates that embryos originating from the right side migrate to the left horn for attachment. The reason for the right-to-left migration, which apparently is unique to Camelidae, is not known. One explanation for this phenomenon implicated a differential luteolytic effect of the left versus the right uterine horn.[38] It was concluded that the right horn effects luteolysis through both systemic and local pathways. An interesting difference was found between the uterine vascular system in the llama and the alpaca and that of animals of other farm species.[40] This was the presence of a major branch of the right uterine artery that crossed the cranial intercornual area to supply much of the left uterine horn. Thus, the vascular anatomy indicated that much venous blood from the left horn drained to the right side; this finding is compatible with the hypothesis that the left horn can exert luteolytic control over the corpus luteum in the right ovary through a local venoarterial pathway. Whatever the cause of migration of the embryo to the left uterine horn, this may be a unique mechanism by which camelids reduce twins to a single fetus.

Multiple ovulations occur in 3% to 10% of alpacas after natural mating and in 9% to 20% after treatment with gonadotropins, but twins born alive are very rare.[41,42] Twin pregnancies are not uncommon in the early stages of gestation but are very seldom observed at late stages or at parturition. Very few cases have been recorded in Peru in alpacas or llamas; a higher rate reported in the United States[42] may be a reflection of better nutrition.

The role of the corpus luteum during pregnancy in the alpaca and llama has been studied,[46] and results indicated that the corpus luteum is necessary for the maintenance of pregnancy throughout gestation in both llamas and alpacas. On the basis of the source of progesterone required to maintain pregnancy, these species may be classified as corpus luteum–dependent, similar to the cow, goat, and sow.

The placenta in the alpaca, as in other camelids, is diffuse and epitheliochorial in type. The chorionic epithelium is thrown into unbranched villi or folds, which are closely apposed to corresponding undulations in the endometrial epithelium. Hence, the fetal-maternal interface consists of an intricate interdigitation of microvilli. In late gestation, both chorionic and uterine epithelia are deeply indented by placental capillaries, so that the intercapillary distance may be as little as 2 µm. The distance appears to be less that that found in the epitheliochorial placenta of any other species of domestic ungulates in late gestation and may be one of the several adaptations to pregnancy at high altitudes.[43] A unique extrafetal membrane has been described in newborn alpacas, llamas, vicuñas, and guanacos.[44,45] The extra membrane encases the entire fetal body and is attached at the mucocutaneous junctions and coronary bands; it appears to be a product of the basal layer of the epidermis.

EMBRYONIC AND FETAL MORTALITY

Diverse studies have established that prenatal loss attains considerable levels in all farm animal species and that the bulk of this loss occurs during the early embryonic stages.

In an early study[31] more than 80% of the ova recovered 3 days after mating were in the process of dividing, and only 50% of the fertilized ova survived for more than 30 days of gestation. In a later study,[47] however, reproductive wastage in a group of alpacas of differing ages and parity was 83% (ova, embryo, and fetus loss). Very few fetal losses were found from 90 days after mating to parturition, and most were late-term abortions.

Factors responsible for this high attrition rate are unknown, but nutritional constraints, hormonal imbalance, improper day of mating and mating system, and chromosomal aberrations may be principal causes. These studies were conducted in alpacas living in their natural habitat, affected by a harsh natural environment, deteriorating feed supply caused by overgrazing, and the presence of infectious and parasitic diseases. Specific infections of the reproductive organs, however, have not been identified as a cause of embryonic losses in llamas.[7] The overall rate and causes of embryo and fetus loss await more critical evaluation.

PARTURITION AND ITS CONTROL

Among 130 alpacas observed giving birth at La Raya Research Station in Cusco, Peru, unassisted labor lasted a mean of 203 ± 129 minutes for primiparous females (n = 34) and 193 ± 122 minutes for multiparous females (n = 96).[39] The time course for the three different stages of labor is indicated in Table 115-3. In llamas (n = 95), a mean of 176 minutes has been reported for the three different stages of labor.[49] Birth in alpacas and llamas appears to be achieved more easily than in the cow or mare. Camelids in general do not lick their offspring at birth, nor do they engage in placentophagia.

More than 90% of births in alpacas and llamas occur between 7 AM and 1 PM. This adaptation gives the cria the best chance to get warm and dry before nightfall, because even in the summer freezing temperatures are common at altitudes higher than 4000 m.[6] In studies done in Australia,[50] the time of parturition in alpacas has been shown to be under photoperiodic control. Also, alpacas appear to be able to delay birthing for hours or even a day to avoid giving birth during the night or during inclement weather.[51] It has been postulated that the fetus may determine the day of birth and the mother determines the hour.[52]

Induction of parturition may be used in farm species to concentrate the birthing season to a confined period. The birthing season in the highlands of Peru lasts about 3.5 months as a result of a prolonged period of mating. In a group of females exposed to males for 60 days, the birthing period lasted 75 days (Sumar, unpublished data). In an early study done in Peru,[53] pregnant alpacas within 45 days of parturition were treated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 0.12 mg, $PGF_{2\alpha}$ 0.25 mg, dexamethasone 10 mg, dexamethasone 10 mg plus estradiol 10 mg, or saline (in control animals). Results showed that both doses of $PGF_{2\alpha}$ significantly shortened the interval to parturition. No undesirable effects were observed during parturition or lactation; however, some excess risk for mortality was found for crias born with a body weight less than 5 kg (normal = 7 kg). The results clearly show the effectiveness of $PGF_{2\alpha}$ for elective induction of parturition. In a more recent study, a prostaglandin analogue, fluprostenol, effectively induced parturition, whereas dexamethasone was associated with fetal death and oxytocin had no effect.[54] In another report,[55] another prostaglandin analogue, cloprostenol, effectively aborted llamas at up to 7 months of gestation without adverse effects on subsequent fertility (250 µg given intramuscularly, to time at 24 hours interval). Of interest, the interval from treatment to parturition or abortion was 21 hours 29 minutes (range, 19 to 29 hours) in alpacas given fluprostenol, whereas in llamas given cloprostenol the interval was 3 days. It is unknown whether differences are due to species, prostaglandin analogue or dose, or the stage of gestation at the time of treatment.

Table **115-3**

Duration of Labor Stages in Primiparous versus Multiparous Alpacas

	DURATION (MEAN ± SD MINUTES)		
Parity	**First Stage**	**Second Stage**	**Third Stage**
Multiparous (n = 96)	87.5 ± 67.2	24.7 ± 16.0	80.7 ± 39.0
Primiparous (n = 34)	101.5 ± 77.6	24.5 ± 12.8	77.0 ± 38.6

Data from Condorena N, Sumar J, Alarcón V: Per yodo de gestacion en llamas *(Lama pacos). Turrialba* XXXX;42:112–113.

POSTPARTUM PERIOD

Up to the fourth day after parturition, the female alpaca is submissive and will allow herself to be mounted by the male.[47] Luteal regression, follicular growth, and uterine involution are not complete, however, and the female will not ovulate; moreover, she is at risk of uterine infection from such early matings. By 10 days post partum, the diameter of the largest follicle is about 8 to 10 mm, the corpus luteum has regressed considerably, and the uterus has involuted substantially (weighing only one fifth of its weight 24 hours after birth). Mating of females within 15 to 20 days after birthing has been recommended to obtain good pregnancy rates, with one offspring per female per year.[56] In a study of llamas, pregnancy rates from breeding at 20 or 30 days were threefold higher (61%) than at 10 days post partum (21%).[10]

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CHAPTER 116

Breeding Soundness Examination of the Male Llama and Alpaca

P. WALTER BRAVO

Breeding soundness examination (BSE) is common in several livestock species and is an excellent tool that can be used to discard males that do not fit selection criteria. In llamas and alpacas, this procedure is still under development; nonetheless, there is tangible evidence that semen evaluation may be done without much elaboration or the use of sophisticated instrumentation. This section does not deal with different traits that are used to select a sire, commonly known among llama and alpaca breeders as a "padre." Other phenotypic criteria are beyond the scope of this section and the reader is counseled to look for further literature. Rather, this section is devoted completely to the examination of the external genitalia and their physiology, including semen evaluation and assessing circulating concentrations of testosterone. Some general criteria for identification are also considered because most BSEs will be used for future sires.

IDENTIFICATION

Most males considered for BSE should have an accurate and lasting identification. Ear tags, collars, and recently, microchips are used to identify animals. Different brands of microchips, ear tags, and collars are available on the

the newborn guanaco *(Lama guanicoe)*. *Theriogenology* 1988;30:437–439.
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CHAPTER 116

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IDENTIFICATION

Most males considered for BSE should have an accurate and lasting identification. Ear tags, collars, and recently, microchips are used to identify animals. Different brands of microchips, ear tags, and collars are available on the

market. Microchips, once properly inserted, last for as long as the animal lives. They are easily inserted beneath the skin in various parts of the body. Most common places are at the base of the ear's pinna. Sometimes, though, the microchip may be inserted at the base of the tail. Owners in the United States use specific names for their animals and usually the name refers to or is correlated to the animal's lineage.

EXAMINATION OF THE EXTERNAL GENITALIA[1–3]

Testicles are a set of paired organs within the scrotum located in the perineal area of the animal. They should be ovoid in shape and resilient to digital palpation. The scrotum is relatively small, nonpendulous, and protrudes from the body outline in a position rather similar to that seen in boars and dogs. The testicles should move freely within the scrotum. Any attachment to the scrotum may signify a previous or ongoing insult to them. The longitudinal and transverse axes of the testicles may be measured using calipers. The testicles increase in size with age (Table 116-1), represent 0.02% of the animal's body, and mature relatively slowly. Ultrasonography of the testicles may be done with the animal in a standing position or in lateral recumbency. The echo texture of the testicle is hypoechogenic with a clear echogenic line in the middle indicating the presence of the mediastinum.

The epididymis may also be palpated, and head, body, and tail portions can easily be distinguished. In llamas and alpacas, the head of the epididymis is bigger than the tail, in contrast to rams and bulls in which the tail is markedly bigger than the head.

The penis has a sigmoid flexure just cranial to the scrotum, and the tip of the prepuce is directed caudally in the nonaroused state. Thus, the stream of urine is directed backward. However, during copulation and with the aroused penis, the prepuce is directed cranially. The penis may also be inspected when the animal is sedated and in lateral recumbency. For this purpose, the sigmoid flexure may be straightened by manual manipulation and the penis may be pushed toward the preputial orifice while the other hand retracts the prepuce. A second person is needed to grasp the penis with a gauze sponge and exteriorize it to check for anatomic abnormalities. In young animals, the prepuce is attached to the penis and this natural attachment disappears with time, driven by the rise in circulating testosterone at 21 to 24 months of age. The glans penis is totally free from any attachment by 3 years of age. The process of detachment begins with the distal tip of the glans.

EXAMINATION OF THE INTERNAL GENITALIA[1–4]

The internal organs that may be evaluated are the prostate and the bulbourethral glands. The prostate has been examined by ultrasonography and has been described as a hypoechogenic mass of tissue located cranial to the bladder. The bulbourethral glands may be also evaluated digitally and by ultrasonography. Digitally, a gloved and lubricated finger is inserted into the rectum and is directed laterally where two well-defined and hard rounded structures are palpated. By ultrasonography, the bulbourethral glands appear as discrete, round structures with a hypoechogenic texture.

TESTOSTERONE CONCENTRATIONS[2]

Testosterone is the main male reproductive hormone. Its presence drives sexual libido and imposes some secondary sexual characteristics. Testosterone is present in basal concentrations from birth through 18 months of age (see Table 116-1). Thereafter, there is a dramatic increase (especially in alpacas), which coincides with appearance of sexual libido as well as disappearance of prepuce-penis attachment. Some males, though, are precocious; they have an accelerated burst of testosterone and consequently an increased sexual drive. On the other hand, some other males have a slow change in testosterone concentrations, which naturally will make an impact on sexual drive. In clinical circumstances when a male is presented for BSE, it is useful to collect a blood sample for testosterone determination to correlate with age, testicular size, and sexual drive.

Table **116-1**

Mean Testicular Size and Testosterone Concentrations by Age in Llamas and Alpacas

	LLAMAS		ALPACAS	
Age (Months)	Testicle Size*	Testosterone†	Testicle Size*	Testosterone†
6	2.4 × 1.4	120	1.0 × 0.4	67
12	3.4 × 2.3	150	2.3 × 1.5	213
18	3.5 × 2.6	140	2.8 × 1.9	1156
24	3.9 × 2.3	500	3.3 × 2.2	2163
30	4.4 × 2.5	600	3.6 × 2.4	2835
36	4.5 × 2.7	800	3.6 × 2.4	5385
Sires	5.4 × 3.3	1000	3.7 × 2.5	5247

*Measured in cm (length × width).
†Measured in pg/ml.

SEMEN COLLECTION[5–10]

Many methods can be used to collect semen and evaluate it. Llamas and alpacas copulate for periods in excess of 20 minutes, and semen deposition is at the level of the uterine horns. In addition, ejaculation is constant and the penis interchanges uterine horns during copulation, which leaves some semen at the level of the external cervical os. Hence, the most practical method to collect semen under clinical circumstances and for BSE is aspiration from the external cervical os after the male has finished copulating. The other reliable method of semen collection is by using an artificial vagina fitted in an alpaca dummy in copulatory position. This method has been widely used in South America and has been instrumental in the definition of some semen characteristics and the initiation of making semen available for artificial insemination. However, it requires training of the male to the dummy. In the case of BSE a male is not trained to an artificial vagina, which leaves the alternative of using the aspiration from the external cervical os. Briefly, a male is presented to a receptive female (a second female should be available in the event that the first is nonreceptive to the male). The male is allowed to copulate for the time he wants and then when he withdraws, the female is restrained. The female's perineal zone is cleansed and a warm lubricated vaginal speculum is inserted into the vagina. The speculum is inserted gently, first upward to pass the vestibule, and then horizontally into the vagina. Once the cervix is reached, a scooping motion of the speculum is made to retrieve as much semen as possible. The vaginal speculum is withdrawn and its contents, a reddish gelatinous material, is transferred into a warmed vial, or alternatively the fluid material is siphoned into a warmed insemination pipette. A drop of the material collected is deposited onto a warmed glass slide, a coverslip is applied, and motility is assessed immediately using a light microscope. The rest of the semen sample may be set aside and kept warm for later evaluation.

SEMEN EVALUATION[6–10]

The appearance of the ejaculate is reddish because of presence of red blood cells as a result of irritation of the endometrium by the copulatory process. Most of the time, the semen collected is gelatinous.

Motility is assessed in terms of the percentage of motile spermatozoa in a microscopic field. Spermatozoa appear to move in a slow manner due to the presence of the gel material in the seminal fluid. In the author's experience, this is perfectly normal for a camelid semen; there is no progressive motility. There are many red blood cells in the sample and spermatozoa may be clumped in certain spots or spread evenly on the slide (Table 116-2). In general, motility may be as high as 70% to 80%, but usually decreases to 0% to 55% during the summer months in the Northern Hemisphere (Fig. 116-1).

Vital staining may also be done using Hancock's stain provided by the Society for Theriogenology. The proportion of live/dead spermatozoa is also affected by season. During the spring, fall, and winter, the percentage of live spermatozoa varies between 70% and 85%, but during the summer it decreases to 0% to 40% (Fig. 116-2). Generally, there are no significant differences in motility and percentage of live sperm between llamas and alpacas (Table 116-2). Sperm morphology may be evaluated by using an assortment of stains. The author has used Giemsa staining as well as spermatic stain (Table 116-2).

Sperm concentration is difficult to evaluate when semen is aspirated from the cervix after copulation. The limiting factor is the unknown volume of semen ejaculated. The sample is set aside for at least 3 hours and then is diluted 1:50 or 1:100 depending upon initial motility

Table **116-2**

Motility and Live Sperm in Different Ages of Male Alpacas

Age	Motility (%)	Live Sperm (%)
Young males (20–26 months)	74	73.3
Three years old	72.2	80
Adult sires	68.5	73.5
Old sires (>11 years)	70	70

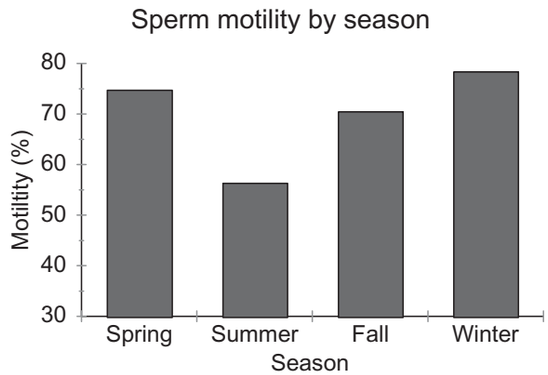

Fig. 116-1 Sperm motility by seasons in the United States.

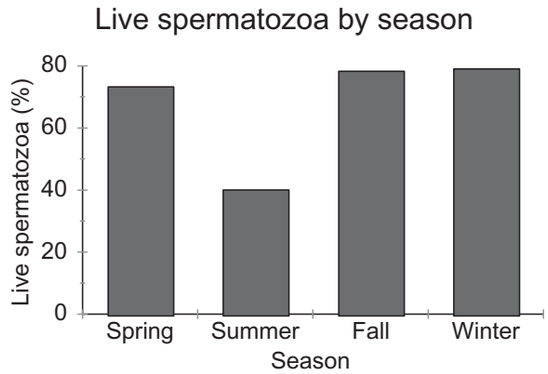

Fig. 116-2 Live spermatozoa by seasons in the United States.

Table **116-3**

Fertility of Young and Adult Male Alpacas

Age	Females Bred	Females Ovulated	Females Pregnant
Young males	45	47%	48%
Adult males	248	70%	69%

assessment. Using the hemacytometer method the author has determined that concentration is the semen characteristic that varies most. On seven different adult males, spermatic concentration varied from 3.5 to 65 million/ml (Bravo, PW, unpublished data).

The age of the male also affects semen characteristics. This is particularly important in North America where some alpaca and llama owners desire to use their males at an earlier age (e.g., 18 to 20 months of age). This is in contrast to South America where males are not used as sires until they are 3 years of age or older. Table 116-3 illustrates the fertility of young and adult sires in the United States. Most important, young males do not impregnate as many females as adult males.

In conclusion, breeding soundness examination in male llamas and alpacas is a tool that may represent a small investment considering the value of the animals and attempts to predict the functionality of the testicles. Anatomy of the different reproductive organs is easily done. Digital palpation and ultrasonography may be used. The most important advance in assessing semen without elaborate and sophisticated instrumentation is semen retrieval from the vicinity of the cervical os. This implies that the male copulates for as long as he wants, there is no interruption, and a sample of semen is available for examination. Motility, numbers of live/dead spermatozoa, and spermatic concentration may be measured, giving the seller, buyer, and clinician a precise and objective indication of semen characteristics.

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CHAPTER 117

Semen Collection and Artificial Insemination in Llamas and Alpacas

WILFREDO HUANCA and GREGG P. ADAMS

Artificial insemination (AI) with frozen semen is one of the most important reproductive technologies in farm animal production and is used worldwide in several species of domestic ruminants including cattle, sheep, and goats. The economic benefit of AI has been well documented as a means of enhancing the genetic potential within a herd. The use of AI in South American camelids would provide important opportunities for (1) genetic improvement of the domestic species (llama and alpaca), (2) preservation of threatened wild species (vicuna and guanaco), and (3) international movement of genetic material. However, difficulties with semen collection, dilution, and cryopreservation have limited the development and use of AI in camelid species.

METHODS OF SEMEN COLLECTION

Several methods have been used to collect semen from camelids, with variable results. It is difficult to collect and handle semen from llamas and alpacas because of the mating posture, duration of copulation (varying from 12 to 50 minutes[1]), intrauterine deposition of semen, and the viscous nature of the ejaculate. Attempts at semen collection in camelids have included the use of condoms or intravaginal sacs, vaginal sponges, electroejaculation, postcoital vaginal aspiration, and fistulation of the penile urethra. The use of intravaginal sacs was first described by Mogrovego,[2] but the procedure was cumbersome, it hindered normal copulation, and it was associated with vaginal injury ostensibly when intrauterine intromission was attempted. An alternative approach was to insert sterile sponges into the cranial vagina, but again, semen collection was poor and the semen that was collected was of low quality and highly contaminated.[3]

In early reports[4,5] electroejaculation was attempted in alpacas and involved the insertion of an electroejaculator probe into the rectum and applying gradually increasing electrical stimulation in a periodic fashion (i.e., 4 to 6 seconds of stimulation followed by 4 to 6 seconds of rest). From 1.1 to 1.8 ml of semen was collected but sperm concentration (range: 1000 to 255,000 spermatozoa/mm^3) and quality were extremely variable, and contamination with urine was common.

The development of an artificial vagina (AV), similar to that used in sheep, was first reported by Sumar and Leyva.[6] The AV included a coil spring to simulate the cervix and was mounted inside a dummy that the males were trained to mount. However, the lack of adequate heating equipment required repeated refilling of the hot water chamber to maintain a stable AV temperature during prolonged copulation. The technique was improved with the use of an electric heating pad wrapped around the AV.[7] Collection of semen by an AV mounted inside a dummy is more reliable than other methods and provides a more physiologic sample of semen[6] (Fig. 117-1). An alternative to a dummy mount is the use of a receptive female; the penis of the male can be directed into an AV during the mount.[7,8] The disadvantage of semen collection with an AV is that the males must be trained to serve the AV; perhaps because ejaculation occurs over such a long time, male llamas and alpacas do not serve an AV as readily as bulls and rams. However, consistent results can be obtained once the males become accustomed to the AV with a dummy or live mount.

The results of semen collection by electroejaculation or with the use of an artificial vagina are summarized in Table 117-1. Note the extreme variability in semen volume and concentration. Results of llama studies appear less variable; the average volume of semen per collection was about 2 to 3 ml and the concentration of spermatozoa was about 1×10^6 to 80×10^6 sperm/ml.[8–10]

SEMEN HANDLING—FRESH AND FROZEN

Effective use of AI requires the dilution and storage of semen, but difficulties in semen collection and handling in llamas and alpacas have been an impediment. The semen of alpacas and llamas is highly viscous[13,18] and makes assessment of sperm concentration, morphology, and motility difficult. Ejaculates vary in color from nearly clear to milky white, depending on the concentration of spermatozoa,[19,20] and a comparatively high proportion of morphologic abnormalities (i.e., 40%) is common.[13] Unlike the progressive motility of sperm seen in other domestic ruminants, only oscillatory movement is seen in the ejaculate of llamas and alpacas.[12,21]

The role of high viscosity of camelid semen is not known, but it may create a type of sperm reservoir[22] or may be important for maintaining sperm viability within

Fig. 117-1 Semen collection in llamas and alpacas using an artificial vagina wrapped in an electric blanket to maintain the temperature at 35° to 40° C and placed within a dummy mount.

the uterus.[23] To facilitate handling and processing semen, attempts have been made to liquefy the ejaculate. In a study designed to test the effectiveness of enzymes for liquefying semen (i.e., collagenase, fibrinolysin, hyaluronidase, or trypsin),[24] collagenase was effective in eliminating semen viscosity within 5 minutes with little or no influence on sperm characteristics. A mechanical technique of liquefying the ejaculate[25,26] involved alternately aspirating and expelling the ejaculate through a needle; the technique effectively liquefied the ejaculate and had little influence on other characteristics of semen.

Little information is available on the use of semen extenders. Motility of llama semen was conserved for 24 hours after collection when diluted with a solution of 30% BSA and 60% glucose and placed in a refrigerator.[8] Dilution with a TRIS–glucose–egg yolk semen extender without elimination of viscosity resulted in poor motility (5%) after 3 hours,[15] but recent results with prior mechanical liquefaction and preservation at 5°C improved motility. Pacheco[27] reported the use of semen diluted with 10% egg yolk and 3% citrate and although no information was given about semen quality after dilution, a pregnancy rate of 60% after AI was reported. In another study,[28] semen collected by AV and diluted with TRIS and EDTA extenders with or without surfactant (Equex STM) was prewarmed to 38° to 40° C and maintained at that temperature until insemination.

ARTIFICIAL INSEMINATION TECHNIQUE: INDUCTION OF OVULATION AND PREGNANCY RATES

The first attempt at AI in llamas and alpacas[1] involved 42 female alpacas inseminated with fresh undiluted semen obtained from two vicunas and four paco-vicunas (cross between male alpaca and female vicuna). Semen was obtained by electroejaculation. Immediately before insemination, the females were mated with vasectomized males to induce ovulation. Semen was deposited in the area of the uterine bifurcation by means of a 35 cm-long plastic catheter guided transcervically by rectal manipulation. Only 1 of 42 artificially inseminated females gave birth.

In a study designed to determine the optimal time for AI after an ovulation-inducing stimulus (i.e., hormone

Table **117-1**

Semen Collection in Llamas and Alpacas

Species	Method of Collection	Volume, ml (Range)	Sperm × 10^6 per ml	% Motility (Range)	% Normal	Reference
Alpaca	Vaginal sac	1.9 (0.4–6.6)	33.0	Low	41	Mogrovego, 1952
Alpaca	Electro-ejaculation	(1.1–1.8)	0.001–2.55	Low	—	Fernandez-Baca, 1965
Alpaca	AV-dummy	1.7 ± 0.2 (0.4–4.3)	—	50.0	—	Garnica et al., 1993
Alpaca	AV-dummy	(0.6–2.7)	0.09–0.2	—	—	Garnica et al., 1995
Alpaca	AV-dummy	1.9 ± 0.4	82.5–250	85 ± 5.2	75.9 ± 2.1	Bravo et al., 1997
Alpaca	AV-dummy	3.0	600	—	—	Sumar et al., 1986
Alpaca	AV-dummy	2.6 ± 1.8	0.06 ± 0.03	49.7 ± 22.6	—	Raymundo et al., 2000
Alpaca	AV-dummy	—	—	68.2	—	Bravo et al., 2000
Alpaca	AV- female	1.8 ± 0.8 (0.6–3.8)	17.6 ± 26.1 (0.05–92.9)	—	51.0 ± 12.4	Flores et al., 2002
Alpaca	AV-dummy	(0.6–8.2)	63–250	55–75	55–75	Huanca et al., unpublished
Llama	AV- female	2.4 (0.2–6.5)	106.8 (15.0–640)	15–50	90.6	Gauly et al., 1996
Llama	AV-dummy	3.0 ± 1.9 (0.2–7.9)	1.0 ± 0.8 (0–3.4)	23.7 ± 20.0 (0–65)	39.7 ± 18.5 (0–79.1)	Lichtenwalner et al., 1996
Llama	AV-female	3.5 ± 2.6	85 ± 89	25–33	32.5 ± 22.3	Von Baer et al., 1999
Llama	AV-female	2.1 ± 1.4	80 ± 28	57.6 ± 22.3	—	Huanca et al., 2001

AV, artificial vagina.

Table **117-2**

Results of Artificial Insemination in Alpacas and Llamas

Species	Route of Insemination	Diagnosed Pregnancy Rate	Birth Rate	Reference
Alpaca	Transcervical	—	1/42 (2%)	Fernandez-Baca, 1968
Alpaca	Transcervical	27/58 (46.6%)	Not reported	Calderon et al., 1968
Alpacas and llamas	Transcervical	37/94 (39%)	Not reported	Leyva et al., 1977
Alpaca	Transcervical and laparoscopy	40/62 (68%)	Not reported	Bravo et al., 1997
Alpaca	Transcervical	105/207 (51%)	Not reported	Apaza et al., 2001

treatment or copulation with a vasectomized male), 96 female alpacas were artificially inseminated 7 to 45 hours after stimulus. Fresh, undiluted semen collected by electroejaculation was infused transcervically by rectal manipulation or through a vaginal speculum.[5] The alpacas were slaughtered 72 hours after AI and the fertilization rate was 12%, 53%, 43%, 75%, and 58% at 7 to 18 hours, 19 to 26 hours, 27 to 34 hours, 35 to 45 hours, and 52 hours after AI, respectively. No differences were observed between groups stimulated to ovulate by hormone treatment or through the use of a vasectomized male.

Leyva and associates[29] reported a study of 83 alpacas and 11 llamas inseminated with fresh undiluted semen collected by electroejaculation from one vicuna and four paco-vicunas. Semen was deposited into the uterine horns, and ovulation was induced by administration of human chorionic gonadotropin (hCG) or mating with a vasectomized male. The pregnancy rate was 48% in females treated with hCG and 11% in females mated to vasectomized males. More recently[13] the pregnancy rate of female alpacas inseminated with fresh semen obtained by AV and deposited into the uterine horns transcervically or by laparoscopy (20 per group) was 73% and 67%, respectively. Similar results were reported by Pacheco[27] using semen diluted with egg yolk (10%) and citrate (3%) using transcervical deposition and induction of ovulation with gonadotropin-releasing hormone (GnRH) 24 hours before AI (Table 117-2). In another recent study,[26] 51% of 207 alpacas were diagnosed pregnant after transcervical insemination using semen collected by AV and mixed 1:1 with BSA (30%) and glucose (60%); ovulation was induced with a GnRH analogue (Busereline; Intervet) 24 to 26 hours before AI.

Reports on the use of frozen semen are limited. Llama semen collected by electroejaculation and diluted with TRIS–egg yolk–glycerol had only 10% motility after freezing and thawing.[30] Ejaculates collected from two llamas and three alpacas by AV were liquefied with collagenase (1 mg/ml) and diluted first with a mixture of sodium citrate (2.9%) and egg yolk (10%) and then with 7% glycerol added at 15-minute intervals in three equal parts before freezing.[31] Sperm motility was estimated at 80%, 60%, and 30% to 40% before freezing, after final dilution and cooling (before freezing), and after thawing, respectively. Most recently,[32] ethylene glycol was used as cryopreservation agent for alpaca semen collected by AV, liquefied by mechanical action, and diluted with skim milk and fructose. Postthaw motility was 30% and highly correlated with estimates of live sperm and the acrosome reaction.

CONCLUSION

Consistent results for semen collection can be obtained with the use of an AV placed within a dummy mount or held adjacent to a receptive female. The semen of alpacas and llamas is highly viscous and difficult to handle, and although it is possible to liquefy the ejaculate with the use of enzymes such as collagenase, fibrinolysin, hyaluronidase, or trypsin, more research is necessary to determine the effects of such treatment on sperm quality. Development of an efficient method of mechanical liquefaction of semen may be a superior alternative. The potential for use of frozen semen for AI in llamas and alpacas is high, but studies are needed to develop effective protocols for freezing semen that preserve post-thaw viability and satisfactory pregnancy rates.

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CHAPTER 118

Ovarian Function in Llamas and Alpacas

GREGG P. ADAMS

South American camelids possess some unique reproductive characteristics generally unfamiliar to veterinarians practicing outside the indigenous zone for camelids, the Andes mountains. Camelids are the only large domestic species that are induced ovulators; females are in virtually constant estrus until successful mating induces ovulation. The term *estrous cycle* must be qualified when used in reference to induced ovulators because a regular cyclic pattern of behavior does not occur as it does in spontaneous ovulators. The terms *receptive* versus *nonreceptive,* and *follicular phase* versus *luteal phase* are more appropriate for communicating "cyclic" changes in camelids. Sexual behavior and copulation time is strikingly different from any other domestic species, gestation is unusually long, and uterine anatomy, placentation, and birthing all have distinctly different characteristics in camelids than any other species. Although the reproductive characteristics of llamas and alpacas appear to be virtually identical, extremely little work has been done directly comparing the two species. As veterinarians, we have been frustrated at the lack of information about the normal reproductive function of camelids. Our inability to distinguish normal from abnormal has made it difficult to diagnose or prognose clinical conditions, or make any meaningful recommendations regarding breeding management. This chapter is intended to focus on the basic aspects of reproductive physiology of llamas and alpacas. Emphasis is placed on ovarian function including follicle and luteal dynamics, and the implications of endogenous patterns on clinical diagnosis, treatment, and reproductive management.

FOLLICULAR DYNAMICS

As induced ovulators, three naturally occurring reproductive statuses exist in llamas and alpacas: (1) nonovulatory, (2) ovulatory but not pregnant, and (3) pregnant. In a study involving ultrasonographic examination of llamas (n = 41; Fig. 118-1) daily for a period of 60 days or longer,[1] ovarian follicle development was found to follow a wave-like pattern regardless of reproductive status (nonovulatory, ovulatory nonpregnant, or pregnant) or lactational status (lactating, nonlactating). That is, a group of follicles begins to grow synchronously, one of which continues to grow and become dominant while the others (subordinates) grow for a short period and then regress. If ovulation is not induced, the dominant follicle eventually regresses as well, and a new wave emerges so that the ovarian "cycle" repeats itself (see Fig. 118-1). The interval between emergence of successive waves of follicles was longer in nonpregnant animals (20 days) than in pregnant animals (15 days), and lactation was associated with a 2.5-day abbreviation in the interwave interval. Maximum diameter of nonovulatory dominant follicles ranged from 9 to 16 mm and was greater, on average, in nonpregnant animals (12 mm) than in pregnant animals (10 mm). Dominant follicles of successive waves are equally as likely to develop in the ipsilateral as contralateral ovary; they do not regularly alternate between ovaries.[1,2]

In a recent ultrasonographic study, the wave pattern of follicle development was also documented in alpacas.[2] The mean (±SEM) interwave interval was 15.4 ± 0.5 days

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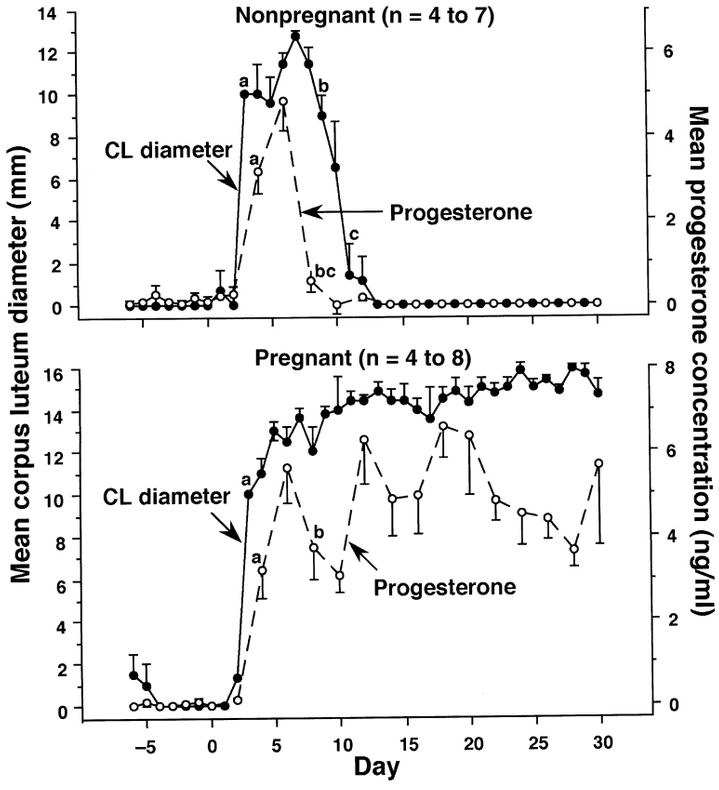

Fig. 118-1 Mean (±SEM) diameter of the dominant follicle of successive waves in llamas of different reproductive status (nonovulatory, ovulatory nonpregnant, and ovulatory pregnant). The arrow indicates the day of mating and the shaded bar indicates the life span of the corpus luteum for the ovulatory groups. (From Adams GP, Sumar J, Ginther OJ: Effects of lactational and reproductive status on ovarian follicular waves in llamas *(Lama glama). J Reprod Fertil* 1990;90:535–545.)

and ranged from 12 to 22 days. Individual alpacas exhibited relatively short as well as longer interwave intervals, and body weight did not appear to be related to the length of the interval. However, the growth rate of dominant follicles was consistent over the first 10 days after emergence and the dominant follicle reached a diameter capable of ovulation by this time, regardless of subsequent interwave interval. The authors concluded that the optimal time of mating might be predicted in alpacas, provided that the emergence of ovarian follicular waves was controlled.

OVULATION

Female llamas and alpacas ovulate only after copulation, or after the administration of hormones with LH (luteinizing hormone)-like activity.[3,4] In an early study designed to determine factors associated with eliciting ovulation,[4] it was concluded that mounting with penile intromission is necessary to induce ovulation, regardless of whether the male is intact or vasectomized. Neural stimuli from penile intromission, treading and clasping of the male's legs on the back and sides of the female, and gutteral humming sounds emitted by the male are thought to influence ovulation. Circulating concentrations of LH start to rise 15 minutes after the start of copulation, peak at approximately 2 to 4 hours, and decrease to basal values by 6 hours after copulation.[5,6] Based on daily ultrasonography,[1,7] ovulation occurred with remarkable consistency (96% of the llamas) by the second day after the first of two matings (4 to 8 hours apart) with either a vasectomized or intact male. The mean diameter of the ovulatory follicle on the day before ovulation was 10 mm and it ranged from 7 to 14 mm. In a more recent study involving ultrasonography every 4 hours,[8] the interval from mating to ovulation was 30.0 ± 0.5 hours (mean ± SEM), and was not different from the interval to ovulation following gonadotropin-releasing hormone (GnRH) or LH treatment.

Interestingly, it appears that a unique ovulation-inducing factor (OIF) is present in the seminal plasma of camelids. In a preliminary study,[9] alpacas were selected (n = 58) when a growing follicle of 8 mm or more in diameter was detected, and were assigned to groups treated with seminal plasma or saline intramuscularly or by intrauterine infusion. Ovulation was detected only in alpacas given seminal plasma intramuscularly (13 of 14, 93%). It was concluded that alpaca seminal plasma contains an OIF that acts via a systemic route. Bactrian camels are the only other species in which an OIF has been reported.[10] The importance of OIF relative to physical stimuli for inducing ovulation during natural mating awaits further investigation.

Unmated females remain in a follicular phase characterized by more or less constant sexual receptivity,[11,12] but sexual behavior does not appear to be related to follicle size because females are sexually receptive with follicle diameters ranging from 5 to 12 mm.[13] However, follicle size is related to circulating estrogen concentrations[5] and ovulatory responsiveness.[14] Females with small follicles (4–5 mm) did not ovulate after copulation, whereas females with follicles 7 mm and larger and in

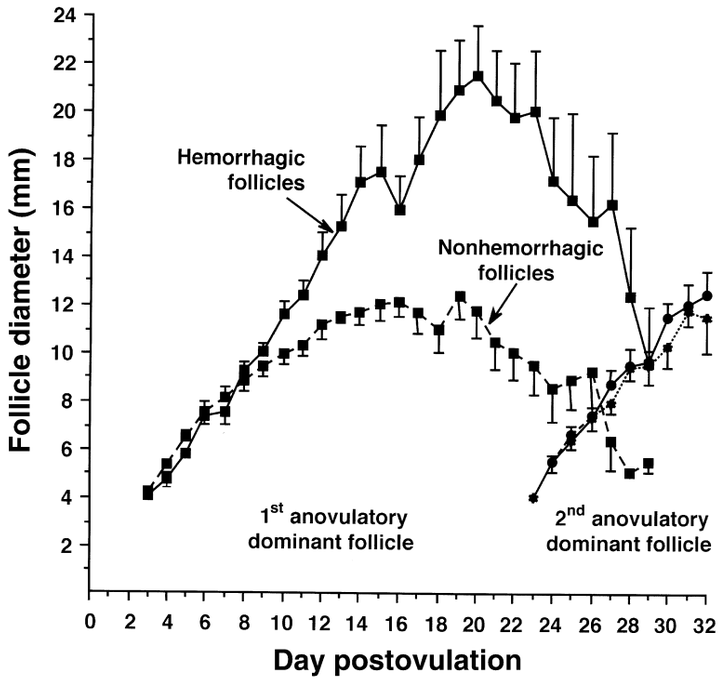

Fig. 118-2 Mean (±SEM) diameter of the corpus luteum and the concentration of plasma progesterone in nonpregnant (vasectomy-mated) and pregnant (intact-mated) llamas from day 6 (day 0 = ovulation) to day 30. (From Adams GP, Sumar J, Ginther OJ: Form and function of the corpus luteum in llamas. *Anim Reprod Sci* 1991;24:127–138.)

the growing phase, or females with mature follicles (8–12 mm) did ovulate after copulation. Regressing dominant follicles did not ovulate after copulation or LH administration.

Even in the presence of a mature follicle, ovulation does not always occur after copulation. Ovulation failure occurs in approximately 20% of pasture-mated females[15] and 10% of hand-mated females.[6] This may be due to inadequate amounts of LH released by the pituitary gland. In this regard, it has been reported that a second copulatory period at 6, 24, or even 48 hours after the first copulatory period did not invoke more release of LH.[6] Correspondingly, an injection of GnRH at 6, 24, or 48 hours after copulation did not invoke more LH release.[6] It appears that if ovulation is to occur at all, it will occur subsequent to the first copulatory stimulus or GnRH/LH administration. The incidence of spontaneous ovulation has been reported to be 4% to 8%.[4,7,16] In some instances, spontaneous ovulation may be associated with the presence of males, owing to visual, olfactory, and auditory stimuli.

LUTEAL DYNAMICS

The characteristics of the luteal phase after natural induction of ovulation have been well described in llamas and alpacas.[1,17–19] Based on daily ultrasonography of the ovaries and every-other-day blood sampling after natural mating in llamas[20] (Fig. 118-2), maximum corpus luteum (CL) diameter and plasma progesterone concentration were detected at day 8 after mating (day 0 = mating). The first significant decrease in CL diameter and plasma progesterone profiles during luteolysis in nonpregnant females occurred on days 11 and 10 after mating and reached their nadir on days 15 and 14, respectively. Similarly, maximum plasma progesterone concentrations occurred on day 8 after human chorionic gonadotropin (hCG) treatment or mating, followed by a decrease beginning on day 13 in nonpregnant alpacas.[14] Luteolysis was temporally associated with pulsatile release of prostaglandin F_2 alpha ($PGF_{2\alpha}$) from the uterus around days 8 to 10 after mating.[17,19]

In pregnant llamas, a transient drop in plasma progesterone concentration was detected between days 10 and 12 after mating, and was followed by a rebound on day 14[20] (Fig. 118-2). Luteal diameter and plasma progesterone in pregnant llamas continued to increase until maximum on days 23 and 27, respectively. It should be remembered that progesterone indicates the presence of a functional CL, but it does not necessarily indicate pregnancy. Elevated concentrations of serum progesterone (>2 ng/ml) or urinary PdG (>1.5 ng/mg Cr) at 7 days after mating are only an indication of ovulation. Furthermore, serum progesterone concentrations of greater than 1 ng/ml at 21 days after mating are only an indirect indication of pregnancy.

CLINICAL IMPLICATIONS

Clinical implications of the endogenous ovarian rhythm involve the timing and control of the follicular wave pattern, induction of ovulation, and control of the luteal phase. At any given time, one may expect to find a follicle of at least 6 mm in one of the ovaries, but to determine whether the follicle is growing (viable) or regressing (dying) would require more than one examination. Such a determination is of importance for breeding management because the immature follicle (<6 mm) and the

overmature (regressing) follicle are not be capable of ovulation and normal luteal development subsequent to copulation. Regarding ovarian synchronization, a recent study was designed to determine the effects of steroids (estradiol plus progesterone [E/P]), gonadotropin (LH), and ultrasound-guided follicular ablation on follicular wave dynamics in lactating and nonlactating llamas, and to determine the effects of these treatments on pregnancy rates after fixed-time natural mating.[21] The intervals from treatment to follicular wave emergence and to the day on which the new dominant follicle reached 7 mm (large enough to ovulate), respectively, did not differ between the LH (2.1 ± 0.3 days and 5.2 ± 0.5 days, respectively) and follicle ablation groups (2.3 ± 0.3 days and 5.0 ± 0.5 days), but both were shorter and less variable than in the control group (5.5 ± 1.0 days and 8.4 ± 2.0 days), while the E/P group (4.5 ± 0.8 days and 7.7 ± 0.5 days) was intermediate. A single, fixed-time natural mating was permitted 10 to 12 days after treatment and although ovulation rates did not differ among groups (control, 93%; E/P, 90%; LH, 90%), the pregnancy rate was higher for synchronized llamas (76%) than for nonsynchronized llamas (54%). The results clearly demonstrate that follicular wave emergence can be induced electively, and animals can be synchronized sufficiently to permit fixed-time insemination without the necessity of testing behavioral receptivity.

OVARIAN IRRREGULARITIES

In general, 15% to 20% of females exhibit some reproductive abnormality.[22] Hypoplastic ovaries, hemorrhagic follicles, cystic follicles, and ovulation failure have been described. Ovarian hypoplasia was the most common ovarian anomaly recorded in a study of slaughterhouse specimens.[22] Hypoplastic ovaries are small (1 × 1.5 cm) and follicular development is suppressed or completely absent. Consequently, estrogen production is impaired and urinary estrone sulfate concentrations are only one third that of normal animals.[23]

The existence of a condition similar to cystic ovarian degeneration, as described in cattle, remains equivocal in camelids on the basis of studies done to date. Based on older literature,[24] follicles greater than 12 mm in diameter were considered "cystic" and therefore subject to hormone treatment to correct the condition. However, based on later ultrasound studies, it appears that cystic follicles in llamas may have been overdiagnosed and overtreated in the past. The normal range in follicle diameter extended to 16 mm and the mean (±SEM) maximum diameter of the dominant follicle was 12.1 ± 0.4 mm.[1] In one study,[25] females that were not exposed to a male (i.e., no sexual stimulation) frequently (16% of nonovulatory follicles) developed oversized follicles (≥25 mm in diameter; Fig. 118-3). These oversized follicles were found to contain bloody fluid and were, therefore, termed *hemorrhagic follicles*. It appears that a vascular accident occurs in some nonovulatory follicles as they near the end of their growing phase, resulting in leakage of blood into the follicle and causing it to balloon to an oversized state. The ultrasonic appearance was characterized as scattered free-floating echogenic spots within the follicular antrum which swirled upon ballottement, similar to that described for hemorrhagic follicles in mares. The antral contents appeared to become organized and did not swirl after follicle growth ceased. Hemorrhagic follicles became very large (up to 35 mm) and persisted for a prolonged period (weeks); however, they resolved spontaneously, they did not disrupt ovarian function, and they were not associated with infertility. Hence, treatment for hemorrhagic follicles does not appear necessary. Clearly, the diagnosis of a follicular cyst cannot be based on size alone and further study is required to critically document the existence of cystic ovarian disease in llamas and alpacas.

Primary ovulation failure has been reported in females that have apparent normal follicle development but do not ovulate after repeated copulation.[23] Attempts to overcome this condition by natural mating or GnRH treatment have been unsuccessful. Although the condition

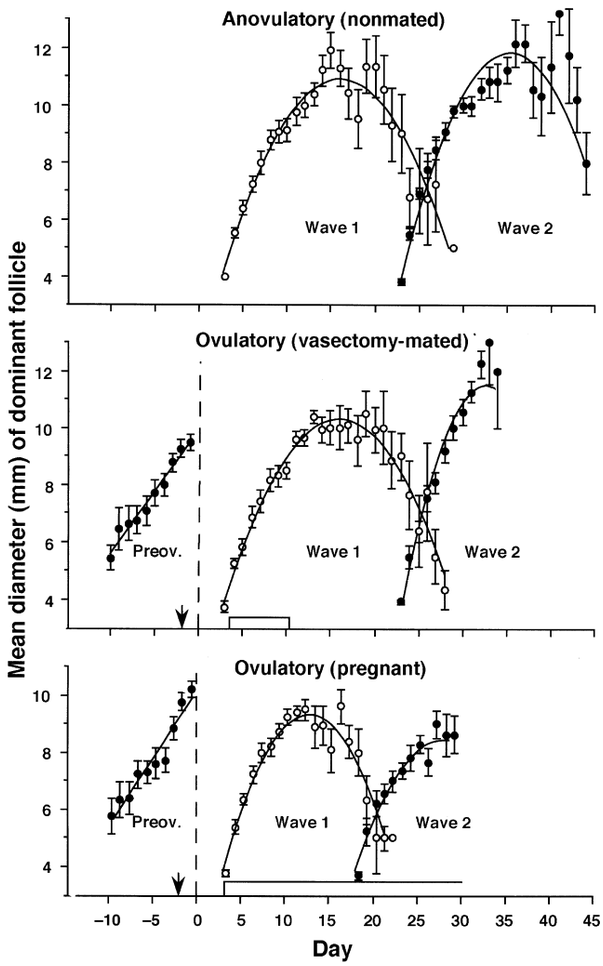

Fig. 118-3 Diameter profiles (mean ± SEM) of successive anovulatory dominant follicles of llamas in which a hemorrhagic follicle was detected *(solid lines)* and llamas in which it was not detected *(broken lines)*. (From Adams GP, Sumar J, Ginther OJ: Hemorrhagic ovarian follicles in llamas. *Theriogenology* 1991;35:557–568.)

has not been systematically studied, it appears that in some cases the pituitary is unable to secrete LH. The obvious solution in such cases is the administration of hCG or LH at the proper time to induce ovulation.[23]

CONCLUSION

Llamas and alpacas have unique reproductive characteristics that differ markedly from other domestic livestock. They are induced ovulators and the ovarian cycle may be conveniently divided into follicular and luteal phases. Follicular waves develop at regular intervals regardless of reproductive or lactational status. The dominant follicle of each wave is anovulatory unless an ovulatory stimulus is provided. Ovulatory stimuli include physical factors, as well as a unique ovulation-inducing factor (OIF) present in the seminal plasma. Ovulation-inducing stimuli trigger LH release from the pituitary within 2 hours of mating followed by ovulation at 30 hours. It appears that a single stimulus is enough to provoke ovulation in the presence of a mature follicle. Circulating progesterone concentrations are correlated with ultrasonographically detected CL diameter. In nonpregnant females, luteolysis begins by day 10 after mating and is complete by day 14, whereas in pregnant females, the CL is maintained throughout gestation. Based on endogenous ovarian patterns, effective protocols have been developed to synchronize follicular waves for fixed-time mating. Ovarian hypoplasia, hemorrhagic follicles, and ovulation failure are the most common ovarian irregularities.

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CHAPTER 119

Breeding Soundness Evaluation and Subfertility in Female Llamas and Alpacas

AHMED TIBARY

Reproductive medicine is most likely the single most common veterinary call in alpaca and llama practice. This is due in part to the difficulty for most breeders to understand the intricacies of reproductive physiology in this species. In addition, the long pregnancy length, the constraint to breed at specific times of the year in order to avoid winter and summer, and the desire to breed to outside males make female reproductive evaluation of paramount importance for the success of a breeding operation. This chapter reviews the standard protocol for the evaluation of the reproductive function in the female lamoids as well as the reproductive disorders and diagnostic approach to infertility.

BREEDING SOUNDNESS EVALUATION OF THE FEMALE LAMOID

Breeding soundness examination of the lamoid female is requested as part of a prepurchase examination or for the diagnosis of the cause of reproductive failure. The protocol adopted in this species is similar to that used for the equine. Routine techniques of examination include history and general physical examination, per rectum palpation and ultrasonography of the genitalia, vaginoscopy, and uterine culture and cytologic examination. In selected cases uterine biopsy, endocrinologic evaluation, hysteroscopy, laparoscopy, and cytogenetic evaluation may be required to reach a diagnosis or prognosis for fertility. Some of these techniques may require sedation (palpation, hysteroscopy), epidural anesthesia (palpation, evaluation of perineal laceration), or general anesthesia (laparoscopy).

History and General Physical Examination

General and reproductive history should cover all parameters that may have an impact on reproductive performance including age, type of animal, and breeding management system. A history of previous illnesses and treatments is important because of their possible effect on reproductive performance, and the information helps identify females with high-risk pregnancies. Complete clinical evaluation of the female, including body condition and actual weight, should be done before any manipulation of the genital tract.

Female behavior provides a relatively accurate and inexpensive means for pregnancy diagnosis.[1] The behaviors most indicative of reproductive status are spitting and attempting to escape, which are consistent with the presence of a corpus luteum.[2] Receptivity on the other hand is not well correlated with ovarian status.[2,3] Absence of rejection of the male (spitting) strongly suggests nonpregnancy.[4]

Per Rectum Palpation and Ultrasonography

Transrectal ultrasonography of the genital tract is performed using a 7.5-MHz or 5-MHz linear transducer. For alpacas, the transducer is mounted on a handle to allow manipulation without inserting the hand in the rectum. Ultrasonography allows a more precise evaluation of follicular and luteal activity within the ovary. In the early stage of the follicular wave, the ovary appears elliptical with several small (2 to 5 mm in diameter) follicles disposed along the periphery in a diadem fashion. Dominance is established when follicular diameter reaches 6 mm. The dominant follicle continues to grow steadily until it reaches its maximal size of 9 to 14 mm in llamas and 8 to 12 mm in alpacas.[5,6] Visualization of the corpus luteum after mating is possible within 3 days.[5] The corpus luteum is less echogenic than the ovarian stroma. It appears as a protruding round structure, sometimes with a central cavity. In other cases, the mature corpus luteum has a dense, hyperechoic central area in the shape of a star. The diameter of the mature corpus luteum varies between 11 and 13 mm.

Pathologic changes of the ovaries detected by ultrasonography include cystic changes (anovulatory or hemorrhagic follicles), inflammation, and tumors. Hypoplastic ovaries are very small and the condition is suspected if ovaries cannot be visualized. In Camelidae, development of large anovulatory hemorrhagic follicles is common in the absence of ovulation-inducing stimulus. Periovarian cysts are not rare and should be differentiated from enlarged oviducts. Ovarian inflammation (oophoritis) is suspected when the ovarian size is normal or slightly increased and does not show clear delineation from the rest of the tissue. Ovarian tumors are suspected when the ovary is enlarged and show an abnormal echotexture with no clear follicular dynamic. The ultrasono-

graphic appearance of the ovary depends on the type of neoplasm.

During the follicular phase, the uterus is contracted and the uterine horns are straight. The echotexture of the uterus is usually heterogeneous and shows increased edema of endometrial folds. During the luteal phase, the uterus is relaxed and homogeneous with a medium degree of echogenicity. Abnormalities can be of two types: abnormal uterine echotexture (endometritis, metritis, neoplasm) and abnormal uterine content (pyometra, mummification, maceration, embryonic death).[7,8] Evaluation of the uterine wall can be facilitated by ultrasonography while the uterus is being flushed.

Vaginal Examination

The vulva is inspected for any discharge or lesions and its size and conformation are evaluated. The size of the vulva in Camelidae is relatively small compared to other species. Females presenting increased size and edema of the vulva should be examined for recent parturition or abortion. Abnormal size and position of the vulva in maiden females may suggest presence of congenital abnormalities or intersexuality. Intersexed animals present ambiguous external genitalia sometimes with a rudimentary penis. The distance from the anal sphincter to the vulvar opening is usually increased and the animal shows urinary difficulties.[9] Copious vaginal discharge beyond 7 days post partum is almost always pathologic. Vaginal discharge is suspected upon observation of dried material on the ventral aspect of the tail or by matting of the wool around the tail and perineal area.

Digital examination of the vestibulovaginal area should be performed on all maiden females (persistent hymen and segmental aplasia) and females with pyometra or hydrometra (vestibular or vaginal adhesions). Open-sided specula are helpful if a biopsy punch or a culture swab needs to be guided into the cervix. The most frequent abnormalities encountered in the vagina and cervix are inflammations or adhesions. Vaginal adhesions are a frequent cause of pyometra or mucometra and inability to copulate.[1] The most frequent development abnormality of the cervix in camelidae is the presence of a double cervix.[1] In some cases a thorough vaginal and cervical evaluation requires the use of a flexible endoscope.

Uterine Cytologic Examination and Culture

Endometritis is a common cause of infertility in Camelidae. It is confirmed by uterine cytology and culture. Samples should be taken from the uterine cavity using a double guarded swab. Swabs are examined routinely for aerobic and anaerobic bacteria, Ureaplasma, Mycoplasma, and fungus.[10] The bacteria responsible for endometritis in camelids are essentially those found in the equine and bovine species. Uterine culture may be negative in many cases of chronic endometritis. In the experience of the author, endometrial samples for cytologic examination are best obtained using a cytobrush instead of a swab.

Uterine Biopsy

Uterine biopsy is used to detect inflammatory, degenerative, or neoplastic changes in the endometrium.[11] The technique is similar to that used in the equine. The cervix can be opened by administration of estradiol (0.5 mg) 6 to 8 hours before biopsy. Larger doses of ECP may produce excessive edema and are often counter-productive. The author prefers to perform all manipulation requiring bypassing the cervix without the use of estrogens. This can be accomplished if examination is done when the female has a preovulatory follicle. Evaluation of the specimen should be done by a person familiar with the normal histologic features of the camelid endometrium. Degenerative changes are mainly due to the presence of periglandular or perivascular fibrosis. In severe cases, nesting with cystic dilation of the endometrial glands or lymphatic cysts is observed. A classification of lamoid endometrial biopsies has been proposed.[11]

Endocrinologic Evaluation

Determination of progesterone levels in blood is probably the most widely used hormone assay in Camelidae.[12] Progesterone levels above 1.5 ng/ml indicate the presence of a functional corpus luteum or luteinized anovulatory follicle. The assay is commonly used to determine occurrence of ovulation in bred females. Pregnancy is suspected if progesterone level remains high in a second sample taken 2 to 3 weeks after breeding. This method of pregnancy diagnosis is relatively precise if breeding history is accurate. Plasma estrogens levels above 10 pg/ml in plasma indicate presence of follicular activity.

Hysteroscopy

Hysteroscopy is easily performed using a 9-mm diameter flexible videoendoscope. The cervical canal and uterine cavity can be evaluated for the presence of adhesions, cystic dilation, or abnormal content. Targeted biopsy may be performed during hysteroscopy.[1,13] The papilla at the uterotubal junction is prominent and easily identified. The most common lesions seen at this level are cysts and inflammation of the papillae.

Laparoscopy and Laparotomy

Laparoscopy is an invaluable technique for the confirmation of lesions suspected by ultrasonography or palpation (ovarian hypoplasia, hydrosalpinx, segmental aplasia, periuterine adhesions, etc.), particularly in alpacas.[1,14–16] Selected cases for these procedures include alpacas with suspected ovarian hypoplasia, ovarian masses, hydrosalpinx, pyosalpinx, ovarian and uterine adhesion, and segmental aplasia of the uterus and uterine tubes. Laparotomy is indicated for oviductal flushing to verify the patency of the uterine tube.

Cytogenetic Evaluation

All Camelidae have the same number of chromosomes (n = 74). Abnormal karyotype has been associated with

different forms of reproductive problems in Camelidae. Cytogenetic studies should be considered when external sexual characteristics are ambiguous or when there is extreme aplasia of the ovary or genitalia. Cytogenetic abnormalities described in camelids include XO, XXX, XXY, XX/XY, and XX sex reversal.[1,9] A minute chromosome has been described in several lamoids, but its significance in infertility is not yet known (L. Buoen and A. Weber, personal communication).

COMMON COMPLAINTS IN THE FEMALE LAMOID

The primary complaints in lamoid infertility seen in the author's practice are repeat breeding (75.6%), early pregnancy loss (18.3%), visible abnormalities of the genitalia (4.9%), and continuous rejection of the male (2.4%).[1] Abortion and stillbirth should be considered as part of the subfertility complex. Arriving at a precise diagnosis of the cause of subfertility or pregnancy loss requires a thorough evaluation that should include history and physical examination as well as a complete evaluation of the reproductive organs.[17] In many cases diagnosis of the cause of infertility may require monitoring the female over at least one reproductive cycle (from follicular growth to mating and pregnancy diagnosis). The objectives would be to answer the following questions: What is the expertise of the breeder? Is the male fertile? Does the female have normal genitalia? Is the female ovulating? Judicious choice of examination technique and interpretation allows obtaining an accurate diagnosis.[1] The diagnostic approach to each of these complaints will vary according to the most likely rule-outs for each complaint. In some cases, the approach will vary, depending on whether the female in question is a maiden female or has previously given birth normally.

Lack of Receptivity to the Male

Female reluctance to breed may be due to pregnancy (first rule-out). Behavioral problems are relatively common and may be associated to dominance or sexual differentiation disorders. Reluctance to breed may be caused by pain during mating.

Persistent corpora lutea are rare in the female Camelidae. However, the condition has been suspected in llamas on the basis of prolonged periods of high progesteronemia without pregnancy.[1,18] Many of these conditions may not be truly "persistent corpora lutea" but rather persistent luteinized hemorrhagic follicle. These structures may respond to prostaglandin F ($PGF_{2\alpha}$), but injection may need to be repeated.

Lack of ovarian activity (anestrus or ovarian hypoplasia) does not necessarily translate to lack of receptivity, as many females with these conditions may continuously accept the male.

Repeat Breeding Syndrome

Repeat breeding syndrome can be due to many factors, some of which are pathologic while others are management errors. The pathologic factors involved in repeat breeding include all conditions that may affect gametes or early embryo survival. Management of reproduction in Camelidae is complicated because the signs of estrus are not very reliable and the timing of breeding in relationship to growth and size of the follicle are very critical for induction of ovulation. Diagnosis of the cause of repeat breeding can be challenging and a diagnosis can be achieved only with a complete and sound approach to the problem.

In normal camelid females, fertilization rates are generally greater than 85%. In a trial on 132 normal alpaca females all but 6 females (95.4%) were pregnant on day 12 after a single mating when the dominant follicle was between 8 and 10 mm in diameter (Tibary, unpublished). Failure of fertilization can be due to management errors, ovulation failure, male infertility, and conditions that may hinder semen/ovum transport or survival.

Survival of spermatozoa is reduced in the case of uterine infection. Fertilization takes place in the lower third section of the uterine tube. Ova transport can be impaired by several pathologic processes in the bursa and uterine tube.

Inability to complete copulation is a frequent cause of fertilization failure. This is due to either lack of or partial intromission without ejaculation. Difficulties in intromission are encountered in the presence of vaginal or vestibular anomalies (i.e., partial or total adhesions or persistent hymen) or discrepancies in size between the male and female. An experienced breeder can easily identify these problems.[1]

Management errors account for a significant number of cases of infertility due to repeat breeding syndrome. Male infertility should be always considered when dealing with the infertile females and is discussed in detail elsewhere in this publication. Management errors pertain mainly to inefficient use of males, breeding at the wrong time of follicular development, or overbreeding. Lamoids may be receptive over a wide range of situation as far as follicular development, and many breeders using in-hand mating systems may be confused as to the proper management of breeding. Given the length of follicular wave (8 to 12 days), it is usually recommended to breed every 7 days until the female spits off the male. Using this schedule one breeding should necessarily fall at the right time (presence of a mature follicle capable of ovulation). Once a female has ovulated, the "cycles" become more predictable and the next opportunity for a female to have a dominant follicle is usually 12 to 14 days after ovulation. Daily breeding is not recommended because it may cause uterine inflammation.

Accurate diagnosis requires monitoring the female over at least one reproductive cycle (from follicular growth to mating and pregnancy diagnosis).

Congenital Problems Leading to Fertilization Failure

Congenital causes of fertilization failure include those conditions that may affect behavior, normal follicular development, or gamete transport.

Intersex

Most intersexed animals will show some degree of male-like behavior, but some may still show receptivity, get bred, and fail to get pregnant. Diagnosis of these conditions is based on thorough external and internal examination of the genitalia.

Ovarian Hypoplasia

Ovarian hypoplasia is a common cause of reproductive failure in maiden females. This condition was reported in 16.8% on postmortem examination of 155 infertile alpaca females.[19] Monitoring ovarian activity by ultrasonography every 2 to 3 days for 10 days allows diagnosis of such disorder. Follicular development may be seen in some females, but the follicles fail to develop to an ovulatory size.[20] The overwhelming majority of the cases seen by the authors were bilateral. Various cytogenetic abnormalities were found in about 40% of the animals. Confirmation of the diagnosis can easily be made by laparoscopic examination.[1]

Segmental Aplasia

Segmental aplasia has been described in various portions of the tubular genitalia (uterine tube, uterus, cervix, and vagina). The most common congenital abnormalities of the uterus in Camelidae are segmental aplasia, uterus unicornis, and infantilism.[1,19] Segmental aplasia can affect any part of the genitalia from the vestibulum to the oviduct. Aplasia of the posterior part of the tubular genitalia (cervix, vagina, or vestibulum) may be associated with mucometra or pyometra. Uterus unicornis may be diagnosed by palpation, ultrasound, or hysteroscopy. Pregnancy is possible, but breeding of animals presenting such a condition is strongly discouraged because of the risk of genetic transmission of the abnormality.[1,21] Segmental aplasia of the uterine tube may require laparoscopy for definitive diagnosis.

Acquired Problems Resulting in Fertilization Failure

Ovulation Failure

Failure of ovulation is a common problem in Camelidae.[20,22] Ovulation failure may be due to inadequate LH (luteinizing hormone) release after copulation.[20] It has been suggested in the Bactrian camel that some males with low fertility have a lower concentration or potency of the GnRH (gonadotropin-releasing hormone)-like ovulation-inducing substance.[23] Assessment of ovulation by ultrasonography or progesterone assay should be an integral part of the reproductive management of the female camelid.

Detection of ovulation failure is based on ultrasound management of breeding followed by progesterone evaluation 6 to 8 days after mating. Induction of ovulation may be enhanced by administration of human chorionic gonadotropin (hCG) (500 to 1000 IU, IV) of GnRH (8 μg to 50 μg IM) following mating.

Ovarian Cystic Conditions

Cystic conditions of the ovaries have been described in llamas and alpacas.[19,20] The term cystic ovaries may not always apply to camelidae because a large proportion (30–40%) of nonmated females develop some form of follicular cyst.[1,18] The role of ovarian cysts as a cause of infertility is not well known. An incidence of 4.7% was reported in a random sample of slaughtered alpacas, while a higher frequency of 8.3% was reported in specimens collected from infertile animals.[19] There are few studies dealing with the endocrinology of cystic conditions of the ovaries. Animals with cystic follicles (>12 mm) show a decrease in estrogen secretion.[20]

Therapeutic approaches used are similar to those described in the bovine and consists of administration of hCG or GnRH followed by an injection of PGF_2 analogues 8 to 10 days later. In some situation of luteinized hemorrhagic follicles, females may become nonreceptive for a variable length of time. Administration of $PGF_{2\alpha}$ analogues may help resolve some of these luteinized structures but may need to be administered on several occasions. Daily administration of progesterone in oil (50 mg) seems to regulate the follicular wave and prevent development of new anovulatory hemorrhagic follicles.

Other Ovarian Diseases

Failure of ovulation despite breeding has also been associated with inflammation of the ovaries (oophoritis) due to an extension of a peritonitis or perimetritis. This condition is characterized by loss of follicular activity because of the presence of adhesions between the ovarian surface and the surrounding tissues including the ovarian bursa, uterine tube, and sometimes extending to include some intestinal loops. Definitive diagnosis is made by laparoscopy.

Ovarian tumors have been reported in infertile lamoids and most are teratomas.[19] A case of bilateral teratoma has been described recently in an alpaca. Unilateral ovariectomy followed by partial ovariectomy has been successful in removing the tumor and restoring fertility.[24] Granulosa thecal cell tumors are rare but have been diagnosed in alpacas and can be detected by their ultrasonographic appearance and elevated serum inhibin concentrations.

Endometritis and Metritis

Uterine infection is the most commonly diagnosed reproductive problem resulting in infertility.[10] Uterine infection should be suspected in animals that have a history of repeat breeding or early embryonic death following at least one normal pregnancy. It is important to consider that endometritis may be secondary to overbreeding resulting from ovulation failure or lack of ovarian activity. In fact, in most of maiden females presented to the author for treatment of endometritis the primary cause of infertility was ovulation failure due to ovarian hypoplasia. Diagnosis of endometritis should be based on clinical examination of the animal (vaginal discharge, uterine wall thickening, presence of intrauterine fluid) and confirmed by uterine culture, uterine cytologic examination, and eventually uterine biopsy. Treatment approaches are similar to those described for the equine and consist of uterine flushing followed by daily infusion of antibiotics of choice for 3 to 5 days. Flushing is accom-

plished by placing a Foley catheter (Fr 12 or 14 for alpacas and Fr 18 for llamas) into the uterus and lavage using LRS or DPBS containing antibiotics. Local infusion of the antimicrobial of choice may be applied using infusion pipette or by placing a ram-horn indwelling catheter into the uterus. However, this author is not in favor of the indwelling ram-horn catheter because of the likelihood of complications from irritation. Systemic antimicrobials for 10 to 14 days may be helpful in managing endometritis and metritis in females if catheterization of the cervix is not easy. Treated animals should be sexually rested for 2 weeks. Minimizing number of mating and breeding during the period of the follicular wave most likely to result in ovulation improves fertility and reduces chance of recurrent endometritis.[10]

Uterine infections may result from postpartum complications such retained placenta, dystocia, and uterine prolapse. Postpartum metritis may have systemic effect on the dam or go unnoticed until breeding. Aggressive treatment with uterine lavage and systemic antimicrobials as well as supportive therapy helps to prevent further complications.

Pyometra

This condition has been reported in lamoids.[1] Pyometra with an open cervix and vaginal discharge is observed primarily in the puerperium period and is due to a postpartum complication (retained placenta, dystocia, uterine prolapse) resulting in delayed involution due to infection and accumulation of fluid. Closed pyometra is usually associated with cervical adhesions or prolonged progesterone therapy.

Other Uterine Disorders

Other reported diseases of the uterus include uterine cysts, uterine abscesses, periuterine adhesions, polyps, and uterine neoplasm.[1,11,19,21] Periuterine adhesions are suspected in llamas if difficulty is encountered when attempting to retract the uterus by palpation per rectum. These adhesions are usually a consequence of peritonitis and may originate from postsurgical complications. Uterine neoplasia (adenocarcinoma) has been described in the llama.[11]

Salpingitis, Hydrosalpinx, Pyosalpinx

The most common disorders of the uterine tube in Camelidae are inflammations or pyosalpinx or hydrosalpinx.[1,19] The enlarged uterine tube can be visualized by ultrasonography. In severe cases, the ovary and ovarian bursa may be involved and adhere to each other. Mucosal cysts and microabscesses are reported but relatively rare. Diagnosis of these afflictions requires endoscopic evaluation of the uterine tube papillae or laparoscopy. Prognosis for all these afflictions is poor if bilateral and guarded if unilateral.

Cervical Diseases

Double cervix with some degree of didelphia (double vagina) has been described in llamas and alpacas.[1,19,25] The major complaints are infertility with a persistent vaginal discharge or repeat breeding. Diagnosis of the condition is made after vaginal examination using a speculum and confirmed by direct examination of the genitalia after ovariohysterectomy.

Cervicitis is due to overbreeding, injuries during parturition, or gynecologic manipulations. Cervicitis is usually associated with uterine infections and vaginal mucopurulent or bloody discharge. Other acquired anomalies of the cervix include cervical adhesions or lacerations resulting from a complication of birth or excessive trauma during manipulation.

Complete Vaginal or Cervical Adhesions

All diagnosed cases of pyometra seen by the author were associated with either cervical or vaginal adhesions or with prolonged progesterone treatments. The main cause of cervical or vaginal adhesions is lengthy or inappropriate obstetrical manipulations.

EARLY EMBRYONIC DEATH

Early embryonic death is commonly reported in llamas and alpacas. Incidence of early embryonic death in these species can be as high as 57.8%.[4,26] Most of the early embryonic loss occurs before day 45, and embryonic loss is suspected when the female becomes receptive again or fails to show signs of advanced pregnancy. Some of the possible etiologies of embryonic death in Camelidae include genetic or environmental factors, corpus luteum dysfunction, and uterine pathology such as infection or fibrosis.

Deficiencies in vitamin A, vitamin E, or selenium have been incriminated in increased early embryo loss. Increased early embryonic death has been associated with early postpartum breeding (before 3 weeks).[27] Reduced ovulation rate and embryonic viability may be seen in females in a negative energy balance due to heavy milk production.

Camelids require ovarian progesterone throughout pregnancy; therefore, any alteration of the corpus luteum function either by disturbance of maternal recognition of pregnancy or implantation and iatrogenic administration of luteolytic drug will result in early pregnancy loss. Progesterone supplementation in the form of implants or injections has been advocated as a method for maintaining pregnancy in females with uterine fibrosis or luteal insufficiency. Although there are several anecdotal reports on the efficacy of these treatments, there are no reports on controlled experiments using this therapy. Preliminary results of experiments in the author's laboratory show that progesterone in oil and hydroxyprogesterone caproate are efficacious in maintaining pregnancy, whereas altrenogest is not. Hydroxyprogesterone caproate may be given at a dose of 250 mg every 2 weeks. If progesterone replacement therapy is used to maintain pregnancy, the dose should be gradually decreased or completely stopped 2 to 3 weeks before due date. It is important to monitor fetal well-being by ultrasonography throughout the pregnancy.

ABORTION

Abortion rate after 100 days of pregnancy ranges from 2% to 15%. Known causes of infectious abortion include toxoplasmosis, chlamydiosis, listeriosis, and leptospirosis. Brucellosis *(B. melitensis)* has been reported in South America. Leptospirosis has been incriminated in some abortion storms in llamas and alpacas. Neosporosis (*Neospora caninum*) has been reported recently as a cause of abortion in lamoids. Diagnosis of the cause of abortion is very frustrating and the approach is similar to principles used in other species. Collection of maternal, placental, and fetal samples may improve the chances in reaching a diagnosis.

Iatrogenic causes of abortion include injection of prostaglandin $F_{2\alpha}$ or its analogues, stressful conditions, and administration of corticosteroids even at small doses. Administration of eye ointments containing corticosteroids has been associated with abortion in the last half of pregnancy.

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CHAPTER 120

Ovarian Synchronization and Induction of Ovulation in Llamas and Alpacas

JANE VAUGHAN

Control of ovarian folliculogenesis in South American camelids would improve efficiency of supervised matings and facilitate the development of assisted breeding in all camelids. Conception due to a single mating may be maximized by inducing emergence of a new dominant follicle at a known time to allow mating to be scheduled when the dominant follicle is capable of ovulating a competent oocyte.

Real-time ultrasonography has allowed investigators to observe ovarian follicular dynamics reliably, to establish the presence of follicular waves, and to determine appropriate times of exogenous hormone treatment in domestic livestock including South American camelids.[1–6] Unfortunately, it is not possible to identify whether a follicle is growing or regressing on the basis of a single ultrasonographic examination, and multiple examinations may be impractical and uneconomical. It would be an advantage to control ovarian follicular growth using exogenous hormones so that a dominant follicle develops at a specific, repeatable time after completion of a treatment protocol, which may reliably be induced to ovulate and release an oocyte of relatively high fertility.

The complex control mechanisms that regulate the development of ovarian follicles through different physiologic stages has delayed development of a simple exogenous hormonal treatment that gives a synchronized new wave emergence in animals regardless of stage of follicle wave at the time of treatment.[7] The antral follicle of spontaneously ovulating mammals has a changing dependency on follicle-stimulating hormone (FSH), luteinizing hormone (LH), and other growth factors as it develops during a follicular wave.[8] The development of sensitive FSH and LH assays in camelids would allow investigators to examine the characteristics of gonadotropin secretion and determine whether the assumptions that FSH and LH work in a similar fashion in induced and spontaneous ovulators are valid. If the assumptions are correct, the response of the ovary to endogenous or exogenous hormones would depend on the stage of follicular development and the physiologic status of the ovaries at the time of treatment.

Maximum fertility in female South American camelids has been proposed to occur when the dominant ovarian follicle is greater than 6 mm in diameter and capable of responding to an ovulatory stimulus, and either growing or mature but not regressing.[2,9] Follicular growth is similar from day 0 to day 10 after new wave emergence in alpacas, regardless of subsequent interwave interval and the newly emerged follicle attains an average diameter of 7 mm, 6 days after new wave emergence.[6] Pregnancy rates are reduced in cattle when aged oocytes are ovulated from dominant follicles that are persistent for longer than 10 days.[10] Reduced fertility may also be associated with oocyte aging in camelids that ovulate when the dominant follicle is postmature.[7]

Removal of the existing dominant follicle may be achieved hormonally in domestic species either by inducing atresia or ovulation. Both mechanisms result in a decline in plasma estradiol and inhibin concentrations followed by a surge in FSH and emergence of a new follicular wave.[7]

INDUCTION OF FOLLICULAR ATRESIA

Estradiol

Exogenous 17β-estradiol and estradiol benzoate have been used in cattle and sheep to transiently suppress plasma FSH concentrations in a dose-dependent manner, thus exerting an atretogenic effect on FSH-dependent follicles early in the follicular wave and provoking synchronous emergence of a new wave.[11–13]

A single intramuscular dose of 0.5 mg or 2 mg 17β-estradiol apparently induced follicle regression and new wave emergence in alpacas regardless of the stage of follicular development, suggesting that estradiol has a role in gonadotropin regulation in this species.[14] However, subsequent studies in alpacas treated at random stages of ovarian follicular development have shown that a single intramuscular injection of 1 mg 17β-estradiol or 2 or 5 mg estradiol benzoate (Cidirol: InterAg), with or without simultaneous injection of progesterone (Progesterone: Jurox), had no effect on follicular wave turnover.[6])

Progesterone

Various doses and delivery methods of progesterone have been tested in camelids in attempts to manipulate follicular waves, based on the observations that dominant follicles in llamas are smaller in diameter, exhibit a shorter interwave interval, and produce less estradiol during the

Table **120-1**

Exogenous Progesterone Treatments Used in Llamas and Alpacas

Reference	Species	Source of Progesterone
Bourke et al. 1992[18]	Llama	(a) endogenous progesterone (corpus luteum) (b) 3 mg norgestamet implant SC for 7 days (c) 2 × CIDR 0.3 g progesterone for 9 days
Correa et al. 1994[19]	Llama/alpaca	12.5 mg progesterone IM once a day for 12 days
Bourke et al. 1995[20]	Llama	(a) endogenous progesterone (corpus luteum) (b) 3 mg norgestamet implant SC for 7 days
Aba et al. 1999[21]	Llama	120 mg MPA intravaginal sponge for 9 days
Cancino et al. 1999[22]	Llama	6 mg norgestomet implant SC 9 days plus 3 mg norgestomet and 5 mg estradiolvalerate IM on day 0
Ferrer et al. 1999[23]	Llama	120 mg or 240 mg MPA intravaginal sponge for 13 days
Aba et al. 2000[16]	Llama	120 mg MPA intravaginal sponge for 9 days
Vaughan 2001[6]	Alpaca	25 mg twice a day, 50 mg once a day, 100 mg every other day, or 200 mg every other day progesterone IM 4–21 days
Chaves et al. 2002[24]	Llama	330 mg progesterone CIDR 8 or 16 days
Aller et al. 2002[25]	Llama	1.9 g progesterone CIDR-B 8 days

CIDR, controlled internal drug releasing; IM, intramuscular; MPA, medroxyprogesterone acetate; SC, subcutaneous.

luteal phase.[2,15,16] These features of follicular growth in llamas in the presence of a corpus luteum may be due to the negative feedback effects of progesterone on LH secretion, as demonstrated in female cattle, in which follicular growth may be suppressed in a dose-dependent manner using exogenous progesterone.[10,17]

Intravaginal Progestagen Devices

Different intravaginal devices such as progesterone-releasing intravaginal devices (PRID), controlled internal drug releasing (CIDR) devices, and sponges have been used to deliver progesterone or progestagen to camelids (Table 120-1).

However, only one study has been conducted on the plasma progesterone levels obtained in South American camelids after device insertion. CIDRs (CIDR: InterAg) containing 330 mg progesterone were used in llamas for 8 or 16 days.[24] Plasma progesterone concentrations were similar to luteal-phase levels in llamas for the first 3 days after CIDR insertion, and subluteal thereafter. Variation in plasma progesterone following CIDR insertion was evident in the llamas studied, possibly due to wide variations in ability to metabolize progesterone rather than variation in amount of progesterone released from the device.[26]

During the intravaginal presence of some PRIDs and sponges, some camelids have ovulated in the absence of mating. These devices have been implicated in inducing ovulation or luteinization by their physical presence, usually at the time of insertion or removal.[21,27,28] Endogenous progesterone production may then potentially compromise exogenous hormone protocols. There have also been problems with device retention. CIDRs were expelled by some llamas, or caused vaginal discharges severe enough to necessitate their removal.[18] PRIDs were expelled by some camels and one failed to retain the device at all.[27] Sponges containing 0, 120, and 240 mg of medroxyprogesterone caused severe hemorrhagic, purulent, ulcerated vaginitis in llamas.[23]

CIDR devices. In a recent study, llamas were each treated with a CIDR device containing 330 mg of progesterone (CIDR-G: InterAg) for 16 ($n = 6$) or 8 ($n = 16$) days.[24] In animals with follicles greater than 6 mm prior to device insertion, a significant decrease in follicular diameter resulting in minima (mean 4–6 mm) between days 5 and 7. Follicular diameter began increasing on day 10 in those animals treated with a device for 16 days, but was not reported in females with a device for 8 days. In 3 of 4 animals with a follicle less than 6 mm at CIDR insertion, follicle size remained small and relatively unchanged in the presence of the device.

Follicular waves generally overlap in camelids,[2] but Chaves and co-workers also demonstrated that a CIDR device inserted into each of 6 llamas for 16 days apparently prevented emergence of a new follicle until day 12 after insertion.[24] This suggested that progesterone may exert a suppressive effect on FSH secretion, but FSH assays need to be improved before this can be shown.

Sponges. Intravaginal sponges containing 120 or 240 mg medroxyprogesterone acetate (MPA) have been used in llamas to synchronize follicle growth with mixed success. Aba and co-workers inserted one 120 mg MPA sponge (120 mg MPA: Syntex SA) for 9 days with 100% ovulation rates in 22 animals treated with the gonadotropin-releasing hormone (GnRH) analogue, given buserelin (Receptal: Hoechst), or mated with a vasectomized or intact male 6 days after sponge removal.[21] Plasma estradiol declined to basal levels 3 days after sponge insertion, then steadily increased thereafter until ovulation induction on day 16. No ultrasonography was performed to monitor follicular growth patterns in response to the progestagen, but 5 out of the 10 females conceived after mating.

Ferrer and co-workers used intravaginal sponges containing 0, 120, or 240 mg MPA in llamas for 13 days and

monitored follicle activity using transrectal ultrasonography.[23] Neither dose of MPA inhibited follicular development as follicles of 7 mm diameter were observed in each group. Every llama in the group in which a 240 mg MPA sponge was used developed hemorrhagic follicles 9 days after sponge removal.[23]

Subcutaneous Progestagen Implants

Subcutaneous implants offer an alternative method of progestagen delivery and avoid the problems of induction of ovulation or vaginal discharges associated with intravaginal devices. Two commercially available types of implant have been used in camelids. The first consists of 3 mg norgestomet in a silicone matrix in which progestagen is released relatively slowly, and the second consists of 6 mg norgestomet in a hydron polymer in which progestagen is released relatively rapidly.[26]

Bourke and co-workers used a single 3-mg norgestomet-silicon implant (Crestar: Intervet) for 7 days in llamas as part of a multiple ovulation and embryo transfer (MOET) program.[18,29,30] Ultrasonography of ovarian structures indicated that recipients showed synchrony of follicular development with donors.[18] They also used a single 3-mg norgestomet silicone implant in llamas for 7 days as part of another MOET program,[20] but follicular development was not suppressed regardless of whether follicles were growing or regressing at the start of progestagen treatment. Reasons for differences in follicular development in the two studies were not apparent.

Cancino and co-workers used a single 6 mg norgestomet-hydron implant (Syncro-Mate-B) for 9 days in llamas combined with an IM injection of 3 mg norgestomet and 5 mg estradiol valerate at the time of implant insertion.[22] Ovarian structures were not observed over the first 7 days of implant placement. A follicle greater than 6 mm was present approximately 10 days after implant removal in 23 of 46 females, 18 of which ovulated in response to treatment with the GnRH agonist buserelin.[22]

Vaughan used one or two subcutaneous 3-mg norgestomet implants (Crestar: Intervet) in alpacas but did not consistently induce regression of the existing dominant follicle nor induce controlled emergence of a new follicular wave, even though implants were used for a longer duration and at a higher dose rate than previous workers had used in llamas.[6,18,20] Maximum follicular diameter was reduced on the day of implant removal and 5 days later in females that received two implants, but follicular waves continued to emerge in the presence of norgestomet. The continuation of follicular waves during norgestomet treatment meant synchronous emergence of a new wave at implant removal was not possible.

Progesterone Injections

The intramuscular administration of 25 mg progesterone (Progesterone: Jurox) twice daily, 50 mg progesterone once daily, or 100 mg or 200 mg progesterone every second day was effective at inducing regression of the existing dominant follicle and preventing new wave emergence in alpacas.[6] Follicles that emerged after progesterone treatment ceased exhibited diameters and interwave intervals that were not different to those of control females. The most practical protocol for ovarian follicular control in female alpacas was provided by injecting 200 mg progesterone intramuscularly on days 0, 2, and 4. This protocol induced regression of the existing dominant follicle and allowed synchronous emergence of a new follicular wave, so that by day 16, all females exhibited a follicle with a diameter capable of ovulation. Following use of the day 0 to 4 progesterone protocol, it was established that females were able to ovulate and conceive when mated on day 16; however, pregnancy rates were not different than those currently achieved by random pen-mating.[6]

A combination of progesterone and estradiol may potentiate the effects of progesterone on LH suppression in camelids as has been observed in cattle.[33] However, 2 mg estradiol benzoate administered alone or at the beginning of progesterone treatment had no effect on follicular regression above and beyond the effect achieved by progesterone treatment alone in alpacas.[6]

Summary of Progestagen Use in Camelids

Norgestomet implants, along with intravaginal progestagen-containing devices such as CIDRs, PRIDs, and sponges (in conjunction with exogenous prostaglandin to remove the endogenous source of progesterone) are used in sheep, cattle, and goats to produce subluteal/luteal plasma progesterone concentrations that suppress pituitary LH production. In turn, LH suppression suppresses follicular growth and estradiol production. Despite these suppressive effects, these implants/devices do not inhibit emergence of follicular waves. Instead, follicular waves continue in their presence, but ovulation is prevented until their removal. In sheep, cattle, and goats, other hormones such as different esters of estradiol and GnRH are used with progestagen treatment to control follicular wave emergence. Upon removal of the progesterone/progestagen implant/device, the existing dominant follicle responds to the increasing LH pulses by producing increasing amounts of estradiol until an LH surge is induced and spontaneous ovulation follows. The purpose of using progestagen-containing devices in alpacas is to provide a simple method to induce regression of the existing dominant follicle regardless of its age, and allow synchronous emergence of a new follicular wave following device removal. However, claims of success in controlling follicular growth or improving fertility in camelids using subcutaneous and intravaginal progestagen devices need to be assessed cautiously. At any given time during nonovulatory follicular waves in untreated alpacas and llamas, one would expect to find a follicle of at least 6 or 7 mm diameter and single random matings produce ovulation rates exceeding 80% and pregnancy rates over 50%.[2,6,15,21,31,32] It appears that progestagen treatment only partially controls follicular development in camelids and does not improve ovulation and pregnancy rates above these levels.

INDUCTION OF OVULATION

An alternative method of removing an existing dominant follicle involves induction of ovulation or luteinization of that follicle to permit emergence of a new follicular

wave. Mating with a vasectomized male, or injection of exogenous GnRH, LH, or human chorionic gonadotropin (hCG) in the presence of a growing or mature follicle 7 mm or more in diameter may be used to induce ovulation 28 to 30 hours after injection in South American camelids.[31,36–38] It appears that the hypothalamus or pituitary gland then undergoes a period of refractoriness for 24 hours following the endogenous LH surge, possibly due to depletion of pituitary LH or down-regulation of GnRH receptors in the pituitary gland.[39]

The GnRH analogue buserelin (Receptal: Hoechst) has been administered intramuscularly at a dose rate of 8 µg (range, 4 to 12 µg) for many years in llamas and alpacas to induce ovulation in the presence of an ovulatory-sized follicle.[20–22,29,32,38] More recently LH (Lutropin-V: Vetrepharm) has been used intramuscularly in llamas at a rate of 2 mg.[38,40]

The dose of hCG (Chorulon: Intervet) to consistently induce ovulation in llamas or alpacas with a follicle considered capable of ovulation ranges from 500 to 750 IU administered intramuscularly or intravenously.[41–43] However, multiple injections of hCG over time should perhaps be avoided. England and co-workers treated female llamas intravenously with doses of hCG ranging from 25 to 500 IU.[44] Females were used one or more times in eight trials in summer and autumn and results indicated that higher doses of hCG (100–300 IU) were required in autumn to elicit ovulation compared with a lower dose (25–50 IU) in summer.[44] Antibody formation in response to repeated use of hCG in cattle has been reported and may have been responsible for the increasing hCG dose required to induce ovulation in llamas used repeatedly by England and co-workers.[44,45]

Failure of ovulation occurs in approximately 10% of females and may be related to timing of administration of the ovulatory stimulus. If the dominant follicle (or that which is subsequently selected as dominant) is immature and lacking LH-responsiveness at the time of stimulus, as in cattle, ovulation failure may result.[46] Others have proposed a lower LH release in females with small follicles due to reduced estradiol priming of the hypothalamus and pituitary.[9] Aging follicles may not respond to an ovulatory stimulus either. In camels, there is a sharp decrease in the effectiveness of a single intravenous injection of GnRH or hCG to induce ovulation when dominant follicles age and exceed 20 mm diameter.[47]

If control of follicular dynamics is to be achieved by induction of ovulation in South American camelids, further studies are required to establish whether growth of the newly emerged follicular wave after induction occurs so that the dominant follicle attains a diameter considered capable of ovulation at a consistent time. In one study, workers observed the emergence of a new follicular wave immediately after ovulation in llamas.[2] The dominant nonovulatory follicle in the first wave after an infertile mating appeared between 1 and 5 days after ovulation, was 7 mm or greater at the time of luteal regression 10 days after ovulation, and reached a maximum diameter about 15 days after ovulation.[2] A subsequent treatment with prostaglandin may be necessary to induce luteolysis at a consistent time following induction of ovulation if the optimal time of mating is determined to be less than 2 weeks after ovulation, as natural luteolysis varies from 8 to 13 days after mating in alpacas and llamas.[3,48]

In alpacas, 100 µg cloprostenol IM given more than 4 days after ovulation induced luteolysis.[49] In llamas, 250 µg cloprostenol IM given 6 to 8 days after ovulation successfully induced luteolysis.[3,20,29,36,40] However, Smith and co-workers report the erratic response of corpora lutea in pregnant llamas to a single injection of cloprostenol (Estrumate: Miles Inc.), with some females requiring two to four injections of cloprostenol to induce luteolysis.[50] Prostaglandin $F_{2\alpha}$ toxicity and death have been reported in llamas following intramuscular use of dinoprost tromethamine,[51] but Smith and co-workers did not observe any adverse reactions using two doses of 250 µg cloprostenol IM 24 hours apart in 53 llamas.[50]

The disadvantages of inducing ovulation as a method of controlling subsequent new wave emergence in camelids at the present time include a lack of knowledge in the timing of new wave emergence, the poor success with hCG in some animals, inconsistent luteolysis in response to a single dose of prostaglandin, and risk of animal toxicity using prostaglandin.

CONCLUSIONS

In summary, progesterone may be used to suppress folliculogenesis in South American camelids; however, further research is required to develop a practical treatment that increases reproductive efficiency by reducing the number of matings per conception and increasing pregnancy rates in comparison to randomly mated females. Induction of ovulation may be a simpler and more effective approach to follicular wave synchronization in camelids, but again, further studies are required to elucidate follicular growth characteristics following induction of ovulation. The ability to predict new wave emergence and subsequent follicular growth following atresia or ovulation of an existing dominant follicle will allow improved efficiency of supervised matings and the development of reliable artificial breeding technologies.

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CHAPTER 121

Pregnancy Diagnosis in Llamas and Alpacas

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Diagnosis of pregnancy in llamas and alpacas can be performed by indirect or direct means. The aim of indirect methods is to distinguish between the follicular versus luteal phase of the ovarian cycle, and usually involves assessment of sexual receptivity using a "teaser" male, or measurement of progesterone concentration in blood or milk. Direct methods involve palpation, ballottement, and ultrasonography of the conceptus and associated fluid. Tactful use of indirect and direct methods of assessing pregnancy status will provide the clinician a broad array of breeding management schemes that may be adapted to suit the needs of the individual producer.

THE EARLY EMBRYO

The early embryo resembles that of other ruminant species (Fig. 121-1), but cells of the morula have a darker cytoplasm, and hatching from the zona pellucida usually occurs before the embryo reaches the uterus. Though initially spherical (at ≤7 days after mating), the hatched blastocyst quickly elongates into a delicate filamentous embryonic vesicle that occupies most of one uterine horn by 10 days after mating (Fig. 121-1). Camelids have a diffuse chorioepithelial type of placenta. The chorionic membrane has unbranched villi that form an intricate interdigitation with corresponding undulations in the endometrial epithelium.

SEXUAL BEHAVIOR AND SYSTEMIC PROGESTERONE

It is convenient to consider sexual behavior in parallel with systemic concentrations of progesterone as indicators of pregnancy status in llamas and alpacas because a causal relationship exists between the two. By inference from the results of a number of studies in llamas and alpacas,[1–6] systemic concentrations of progesterone are inversely related to sexual receptivity. That is, females under the prevailing influence of progesterone are nonreceptive to sexual advances of a male.

In an often-cited study of the sexual behavior of alpacas,[6] it was stated that estrus may last for 21 to 36 days with occasional short periods (≤48 hours) of nonreceptivity. In a more critical study in llamas,[3] however, periods of constant estrus of at least 30 days were observed (end of observational period), but extended to at least 90 days in some individuals. No mention was made of short periods of nonreceptivity, other than a prolonged period of diestrus. In nonmated females, follicular waves emerge at regular periodic intervals without interruption by ovulation or luteal gland development.[7] Circulating concentrations of estrogen wax and wane in correspondence with growth of the dominant follicle of each wave;[8] however, no obvious relationship between estrogen concentration and sexual receptivity has yet been detected.[8] The relationship between follicular growth, estrogen concentration, and sexual receptivity has not been quantitatively evaluated and warrants further research, but it is clear that in the absence of progesterone, female llamas and alpacas are more or less receptive continuously.

Based on the assumption that sexual non-receptivity is a reflection of elevated systemic progesterone concentration, the strategy for testing receptivity is similar to that for testing systemic progesterone concentration as an indicator of pregnancy status (Fig. 121-2). Ovulation

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CHAPTER 121

Pregnancy Diagnosis in Llamas and Alpacas

GREGG P. ADAMS and MIGUEL DOMÍNGUEZ

Diagnosis of pregnancy in llamas and alpacas can be performed by indirect or direct means. The aim of indirect methods is to distinguish between the follicular versus luteal phase of the ovarian cycle, and usually involves assessment of sexual receptivity using a "teaser" male, or measurement of progesterone concentration in blood or milk. Direct methods involve palpation, ballottement, and ultrasonography of the conceptus and associated fluid. Tactful use of indirect and direct methods of assessing pregnancy status will provide the clinician a broad array of breeding management schemes that may be adapted to suit the needs of the individual producer.

THE EARLY EMBRYO

The early embryo resembles that of other ruminant species (Fig. 121-1), but cells of the morula have a darker cytoplasm, and hatching from the zona pellucida usually occurs before the embryo reaches the uterus. Though initially spherical (at ≤7 days after mating), the hatched blastocyst quickly elongates into a delicate filamentous embryonic vesicle that occupies most of one uterine horn by 10 days after mating (Fig. 121-1). Camelids have a diffuse chorioepithelial type of placenta. The chorionic membrane has unbranched villi that form an intricate interdigitation with corresponding undulations in the endometrial epithelium.

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Based on the assumption that sexual non-receptivity is a reflection of elevated systemic progesterone concentration, the strategy for testing receptivity is similar to that for testing systemic progesterone concentration as an indicator of pregnancy status (Fig. 121-2). Ovulation

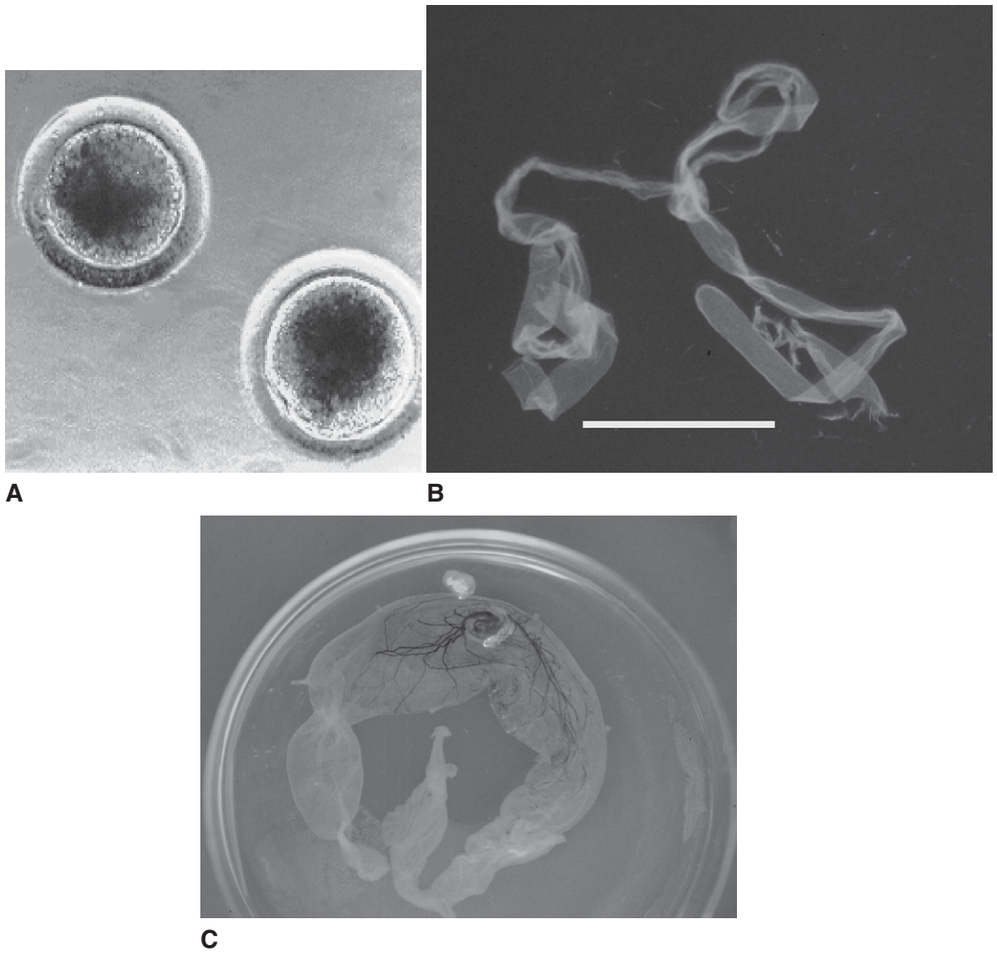

Fig. 121-1 Embryos collected from llamas 3 days after mating **(A)**, 10 days after mating (**B**; scale bar = 1 mm), and 24 days after mating **(C)**. Specimen in a glass Petri dish.

occurs very consistently 1 to 2 days (mean, 30 hours) after the first mating (see Chapter 118), but plasma progesterone does not rise significantly until 4 days after mating.[2] Plasma progesterone concentration peaks 7 days after first mating and decreases rapidly thereafter in nonpregnant animals to a nadir at 11 days after first mating. During the first 3 to 4 days after mating, when the corpus luteum (CL) is forming and progesterone concentrations are low, some females remain receptive to the male. In pregnant animals, plasma progesterone concentration will remain elevated until the last 2 weeks of gestation, at which time it begins to decline to a nadir 24 hours before parturition.[9] In one study, plasma progesterone did not exceed 0.4 ng/ml in nonmated nonovulatory llamas, but it remained in excess of 2 ng/ml in all pregnant llamas after day 11 after mating.[2] In the same study, CL diameter assessed by transrectal ultrasonography was highly correlated with plasma progesterone concentration (r = 83%; $P < 0.0001$) with the caveat that progesterone decreased 1 to 3 days before CL diameter decreased, at the time of luteolysis (see Fig. 122-2). Interestingly, a decrease in mean plasma progesterone concentration was detected between 9 and 11 days after mating in pregnant llamas, as well as subtle decrease in luteal diameter.[2] The transient drop in progesterone was coincident with the initiation of uterine-induced luteal regression in nonpregnant animals and it was suggested that the rescue and resurgence of the corpus luteum between 9 and 1 days after mating represents luteal response to pregnancy (or maternal recognition of pregnancy).[2] Measurement of progesterone concentration in the milk of pregnant and nonpregnant llamas and alpacas during the first 14 days after mating also revealed a parallel relationship with the concentration of progesterone in the blood.[10]

Based on these observations, a diagnosis of ovulation may be made between 4 and 8 days after mating by (1) behavior assessment, (2) measurement of progesterone concentration in blood or milk, or (3) measurement of CL diameter. A diagnosis of *nonpregnancy* may be made by day 11 after mating by detecting behavioral receptivity or basal progesterone concentrations and a regressing CL (see Fig. 121-1).

Test of sexual receptivity is an indirect test of the presence of progesterone, and measurement of systemic

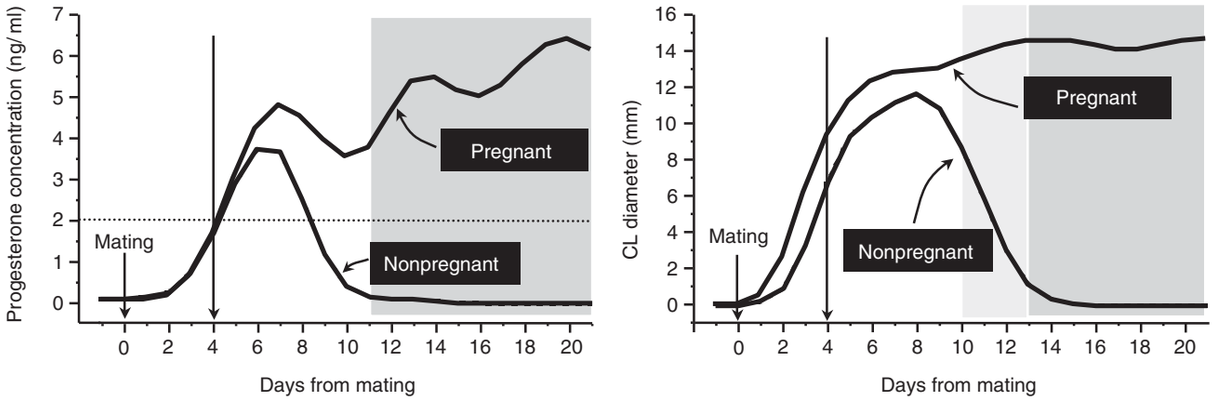

Fig. 121-2 Plasma progesterone concentration and corpus luteum (CL) diameter in nonpregnant and pregnant llamas. The short arrow indicates time of mating, and the long arrow indicates the day of first significant increase in progesterone (day 4). The light shaded area indicates the period when mean CL diameter is significantly different between statuses, and the dark shaded area indicates the period when individual test results will consistently distinguish between nonpregnant and pregnant animals, assuming that pregnancy is the cause of luteal maintenance. Pregnant llamas usually have plasma progesterone concentrations in excess of 2 ng/ml (horizontal dotted line). (Adapted from Adams GP, Sumar J, Ginther OJ: Form and function of the corpus luteum in llamas. *Anim Reprod Sci* 1991;24:127–138.)

progesterone is a measurement of luteal function—*neither* are direct indicators of pregnancy. Hence, behavior assessment and progesterone testing may be more appropriately referred to as methods of diagnosing *nonpregnancy* because luteal gland function may be elicited by factors other than pregnancy. Spontaneous ovulation has been reported in approximately 10% of females not exposed to a male[5,7,11] and may result in a false positive diagnosis of pregnancy. Pathologic processes such as luteal cysts,[4,12] embryo/fetal loss,[1] and abnormalities of the tubular genitalia may result in a prolonged luteal phase and a false positive diagnosis of pregnancy. In one llama that was ultrasonically monitored daily after embryonic loss, luteal regression had not occurred by the end of the observational period (70 days; G.P. Adams, unpublished). Based on repeated ultrasonographic examinations and final inspection at the time of ovariohysterectomy, a clinical diagnosis of a persistent corpus luteum was made in one llama with closed pyometra (cervical adhesions) and in another llama with congenital segmental aplasia and mucometra (G.P. Adams, unpublished). In both instances, the animals were assumed to be pregnant based on behavior and repeated measurement of plasma progesterone during the preceding months. Plasma progesterone concentrations remained above 2 ng/ml, and were unusually high (8 ng/ml) in some samples. The pathogenesis of these irregularities has not been explored and the predictive value of subnormal or supranormal levels of progesterone is unknown. Depending on the range of normal values established by the respective laboratory, progesterone values between 1 and 2 ng/ml are equivocal and warrant repeated measurement.

In a study of the accuracy of behavior testing as an indicator of pregnancy status,[13] the proportion of correct diagnoses ranged between 84% and 95% in llamas and alpacas at 70 to 125 days of gestation. Although convenient and useful, assessment of sexual behavior is confounded by its subjectivity. Submissive behavior in llamas and alpacas is similar to receptive behavior; aggressive behavior in a male or timid behavior in a female may result in submission to mating and mask nonreceptive behavior. Conversely, an inexperienced male may be easily intimidated by a dominant female, and occasionally, inexperienced females refuse to adopt a prone position and need to be restrained for mating. Although behavior and progesterone testing can be extremely useful management tools, they must be used strategically and only for presumptive diagnosis of pregnancy.

OTHER ENDOCRINE INDICATORS OF PREGNANCY

In a study to assess potential endocrine indicators of pregnancy, urine and blood samples were collected throughout pregnancy from 8 llamas and 11 alpacas to measure estrone sulfate and relaxin concentrations.[14] Estrone sulfate concentration in urine and serum peaked twice during pregnancy; at 21 days after mating and again during the last month of gestation. It was concluded, however, that the use of estrone sulfate concentration to diagnose pregnancy is highly dependent on the time of sampling. Measurement of serum relaxin concentration was considered a superior indicator of pregnancy after the second month of gestation because it represents an interaction between mother and fetus (pregnancy-specific) and concentrations were greater than basal values for the majority of gestation.

PALPATION AND BALLOTTEMENT

Scientific reports on palpation and ballottement as methods of diagnosing pregnancy in llamas and alpacas

are scarce. Only one study was found in which an attempt to critically document the accuracy of transrectal palpation was made.[13] Transrectal palpation was possible in 20 of 20 llamas and in 41 of 50 (82%) of alpacas; the rectum of 9 alpacas (18%) was too small to permit palpation. A correct diagnosis was made at 165 days after mating in all animals large enough to permit palpation (100% accuracy). With experience, transrectal palpation can be used to detect pregnancy as early as 35 days after mating, based on marked asymmetry of the uterine horns (conceptus is invariably maintained in the left horn), fluid fluctuance, and in later gestation, palpation of fetal parts. The placenta has a diffuse epithelio-chorial attachment; therefore, a fetal membrane slip characteristic in cattle cannot be felt. No specific reports on the use of transabdominal ballottement were found, but apparently some Peruvian herdsmen are quite skilled and it is used quite commonly after 8 months of gestation.

DIAGNOSTIC ULTRASONOGRAPHY

Two modalities of diagnostic ultrasound have been used for pregnancy diagnosis in llamas, A-mode (amplitude modality) and B-mode (brightness modality). The utility of A-mode ultrasonography for determining pregnancy status was investigated in one study[13] in which alpacas (n = 50) and llamas (n = 20) were examined at 10-day intervals from 70 to 160 days after mating. The transducer was placed in the sparsely haired inguinal region cranial to the udder on either side of midline. Accuracy was 90% to 95% initially but dropped to approximately 65% to 70% after 145 days. In a direct comparison, the accuracy of A-mode ultrasonography for detecting pregnancy was not different from that of behavior testing, but was significantly lower than that of palpation.[13]

With the availability of portable, real-time scanners designed for on-farm use, B-mode ultrasound imaging has become a common tool in veterinary practice. A thorough understanding of anatomy and the principles of ultrasonography are required, but practical capability can be achieved rapidly. For imaging the reproductive organs, the transducer may be placed on the outside of the body and directed dorsally or obliquely across the abdomen (transabdominal approach) or placed within the rectum and directed ventrally (transrectal approach). The transabdominal approach is useful for diagnosing pregnancy after approximately 60 days after mating and was used to generate a graph for estimating gestational age based on biparietal diameter of the fetus.[15] However, the fiber, skin, fat, and muscle of the body wall, and the depth of penetration required causes attenuation of the ultrasound signal and limits the resolution of images.

The transrectal approach involves the insertion of a gloved hand and transducer into the rectum. Because the reproductive organs are located immediately beneath the rectum with very little intervening tissue, the transrectal approach offers better access and a better image than the transabdominal approach. A clear image of the ovaries, uterus, and cervix can be achieved at any time during pregnancy or nonpregnancy. Although the transrectal approach is preferred, some llama and alpaca owners as well as their veterinarians are hesitant to use it. Indeed, physical limitations (i.e., relatively small rectum) may preclude this approach in some individuals. The author estimates that over 90% of mature llamas and over 75% of mature alpacas are able to accommodate the transrectal approach (glove size, $7^1/_2$). A rigid or semirigid probe extension may be used to permit external manipulation of an intrarectally placed transducer in individuals too small to accommodate the operator's hand. Transrectal ultrasonography is a safe procedure, but as with any other diagnostic procedure, it has attendant risks. The most serious risk is trauma or perforation of the rectum, which must be handled as a surgical emergency.[16] Although not critically studied, the incidence of trauma as a result of transrectal examination is apparently no greater in llamas and alpacas than in other large domestic species in which the procedure is routine (e.g., horses and cattle).

The ultrasonographic appearance of the conceptus at different stages of pregnancy, and growth characteristics of the embryo are shown in Figures 121-3 and 121-4. In an ultrasonographic evaluation of 60 llamas during the first 60 days of pregnancy[17] there was no difference in the proportion of left-sided versus right-sided ovulations, but serial examinations revealed that the early embryonic vesicle could be detected in any segment of either uterine horn—ipsilateral or contralateral to the side of ovulation. After day 20, however, the embryo appeared to become fixed in place and was most frequently observed in the middle portion of the left uterine horn, where development continued. After 26 days of pregnancy, the developing embryo/fetus was invariably observed in the left uterine horn (60 of 60 pregnancies). After day 45 the fetus appeared to be laying on its side on the ventral wall of the uterine horn with its head pointing toward the tip of the left horn while the umbilical cord extended toward and sometimes through the uterine bifurcation. In very few instances was the fetus observed hanging from the top of the uterine horn.[17] The embryonic vesicle was first detected as an irregular collection of fluid as early as 12 days after first mating (2 to 4 mm in diameter), and the beating heart of the embryo was first detected by 26 days after mating. In an ultrasound study of 19 alpacas, the embryonic vesicle was first detected in 30% of pregnancies at 10 days after mating, in 50% at 15 days, and in 100% at 23 days.[18] In another ultrasound study of 40 alpacas, the embryonic vesicle was first detected in 50% of pregnancies at 12 days after mating, in 83% at 14 days, and in 100% at 16 days.[19] A reasonable time for initial pregnancy diagnosis, therefore, is about 16 days after mating, depending on examination conditions and the experience of the clinician.

TWINNING AND EMBRYO LOSS

Double ovulation and twin pregnancies do occur in llamas and alpacas, but the birth of twins is rare. Double ovulation was detected in 1 of 83 ovulations (1.2%) in a Peruvian group of llamas, and 5 of 312 (1.6%) in a North American group of llamas.[17] Of the 6 llamas with double ovulations, 5 developed twin pregnancies, 3 singletons were born, and no twins were born. Information about changes in the size and morphology of the developing conceptus is sparse; however, preliminary studies have

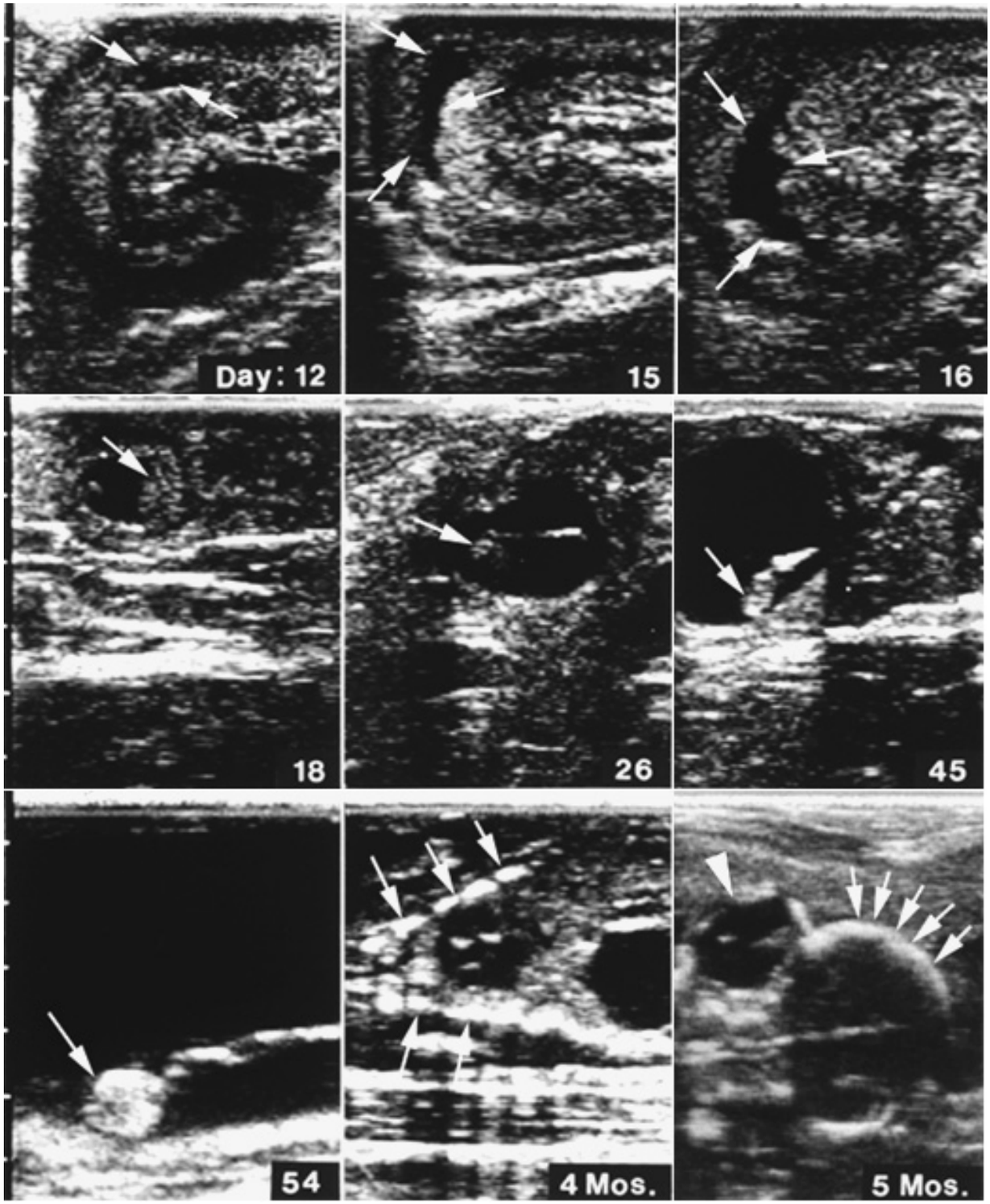

Fig. 121-3 Ultrasonic images of pregnancy in llamas at different stages of gestation (day 0 = ovulation). Note the fluid *(black)* of the embryonic vesicle in the longitudinal sections of the curled uterine horn (days 12, 15, and 16), and the embryo proper *(arrows)* in the cross sections of the uterine horn (days 18, 26, 45, and 54). The fetal chest is recognized by the ribs *(arrows)* and the heart (4 months), and the fetal head *(arrows)* is shown (5 months) with the fluid-filled eye uppermost *(arrowhead)*. (From Adams and Domínguez, unpublished data.)

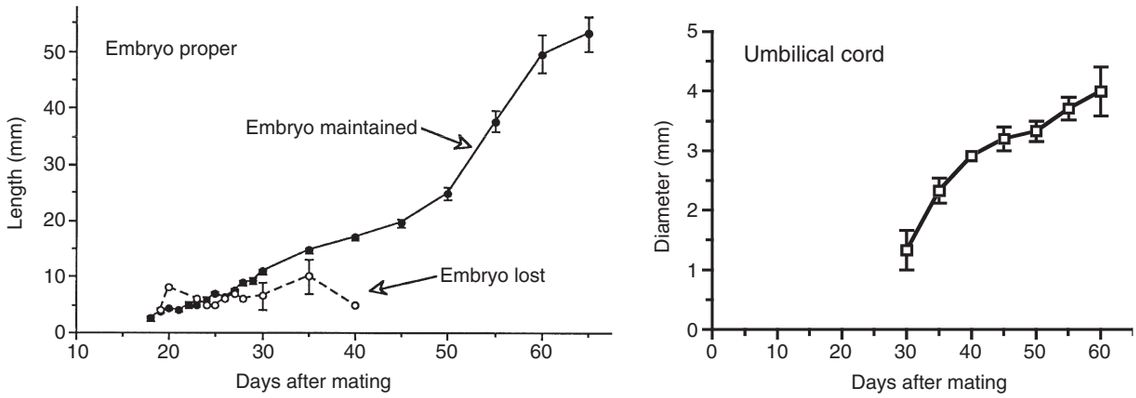

Fig. 121-4 Growth (mean ± SEM) of the embryo proper and the diameter of the umbilical cord during the first 65 days of gestation in llamas (n = 60). (From Adams and Domínguez, unpublished data.)

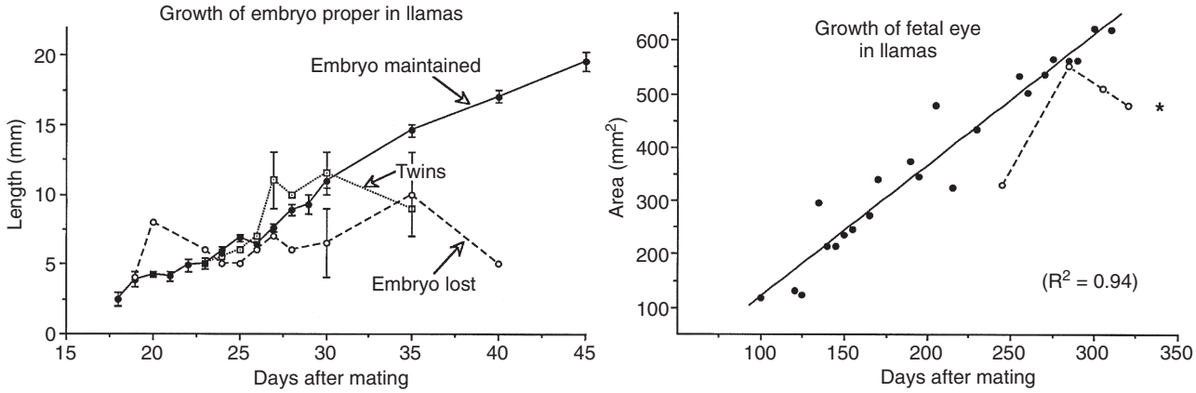

Fig. 121-5 Growth (mean ± SEM) of the embryo proper *(left)* and the fetal eye *(right)* in llama conceptuses that were maintained *(solid line)* and those that were lost *(dashed line)* during gestation. (From Adams and Domínguez, unpublished data.)

yielded data on the crown-rump length, dimensions of the fetal eye, twinning, and pregnancy loss (Figs. 121-4 and 121-5).

The nature and rate of pregnancy loss in llamas and alpacas has not been well documented. In an ultrasound study, 83 pregnancies resulted from 123 ovulations (67%) after a single mating session (two matings, 4 to 8 hours apart).[17] Eleven percent of llamas diagnosed as pregnant by ultrasonography at 20 days or sooner lost the conceptus by 40 days after mating, and no further losses were recorded up to 60 days. It seems reasonable, therefore, to monitor the developing conceptus more frequently during early pregnancy (e.g., days 25, 40, and 60) and less frequently thereafter (e.g., at 3, 6, and 9 months and at term).

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CHAPTER 122

Eutocia, Dystocia, and Postpartum Care of the Dam and Neonate

PATRICK LONG

IMPENDING PARTURITION

Impending delivery in camelids is difficult to discern. External signs normally seen in other species are not readily apparent in camelids. There are minimal changes in udder development and virtually no vulvar relaxation discernible prior to delivery. In addition, there is a great deal of variability in gestation length, ranging from 330 to 365 days in both llamas and alpacas. In a study of the effects of season on gestation length,[1] fall mating was associated with a gestation length 12 days shorter than spring mating. This may be an evolutionary trait to ensure that the crias will be born in the summer season and thus have the greatest chance of survival. An unusual feature of the domestic and wild species of South American camelids is that most births (>90%) occur between sunrise and mid-day.[6] This is thought to be an evolutionary process to ensure neonatal survival. Females starting first-stage labor late in the afternoon warrant careful examination because a high percentage will be dystocias.

NORMAL PARTURITION

As in other species, parturition is divided into three stages. First stage of labor should not exceed 4 to 6 hours, second stage of labor should not exceed 30 to 45 minutes, and the third stage of labor (characterized by delivery of the placenta) should be completed by 4 to 6 hours.

Typical first-stage labor signs are restlessness, loss of appetite, increased humming, and frequent trips to the dung pile with or without urination or defecation. Many times these signs are subtle and will be missed by all but the most observant owner. Increased rolling or lying in lateral recumbency may be noted during this time. First-stage labor lasting more than 4 to 6 hours warrants rectal or vaginal examination.

Second-stage labor is marked by the appearance of fetal body parts, discharge of amniotic fluid, or appearance of placenta. This stage should progress very rapidly and normally will not exceed 30 to 45 minutes. If the cria is not delivered within 45 minutes of the initiation of second-stage labor, intervention is indicated. Because of the long

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slender body of crias, dystocias from an oversized fetus are rare. As in other ruminant species, the normal presentation is anterior in a dorsosacral position with extended forelimbs. The nose and feet appear almost simultaneously. The chest is the biggest part of the cria, and second-stage labor may appear to stop at this point. Females that stop labor at this point may need assistance if labor doesn't progress within 5 minutes. Females will alternate between standing and lateral recumbency, and final delivery of the fetus may be accomplished in either position. Crias are born with a thin epidermal membrane that does not interfere with breathing or mobility.[3]

Delivery of the placenta, or third-stage labor, is usually completed within 6 hours. Female llamas and alpacas may show abdominal distress until the placenta is passed and may be reluctant to allow the cria to suckle until passage of the placenta. Lochia is commonly found by owners on or near the dung pile for 7 to 10 days post partum. This discharge is reddish brown, is gelatinous, and has no odor.

Retained placentas are uncommon and generally treated if not passed within 24 hours. Treatment consists of injections of oxytocin (5–10 units) at 10-minute intervals with or without gentle traction.[4] Strenuous traction may induce uterine prolapse. If the placenta cannot be delivered with this technique, uterine flushes with dilute iodine solutions may be used to facilitate passage of the placenta.

DYSTOCIA

Uterine torsion, breech or posterior presentation, and abnormalities of posture constitute most of the causes of dystocia in llamas and alpacas. Although the reported incidence varies, most authors cite an approximate 5% dystocia rate.

Uterine Torsion

Uterine torsions may occur as early as 7 to 9 months of gestation, but most occur during the last month. Behavioral signs of uterine torsion are often confused with that of gastrointestinal disorders.[2] Severe abdominal pain, violent rolling, and general restlessness are the typical presenting signs for uterine torsion. Causes of uterine torsion are unknown, but factors such as genetic predisposition, rolling, right side ovulation, increased fetal size, and increased fetal activity have been hypothesized.[4] Diagnosis of uterine torsion may be made with a thorough history of breeding dates, a complete physical examination, and rectal or vaginal examination. In a recent report,[2] 95% of torsions in llamas and alpacas were clockwise (or to the right when viewed from the rear). This is consistent with the author's experience with over 40 cases of uterine torsion.

A complicating issue in alpacas is that rectal examination may not be possible because of their small size. Depending on the location of the torsion, a vaginal examination may lead to the diagnosis. Rectal examination will be necessary to diagnose a torsion cranial to the cervix. When palpating the broad ligament, in a clockwise or torsion to the right, the ligament on the right side will be pulled ventral and medial. The ligament on the left side will be pulled to the right and over the top of the uterus. Palpation of these ligaments will cause pain and noticeable discomfort to the dam. Conversely, a torsion to the left or counterclockwise will result in a reversal of position of the ligaments. Vaginal examination will permit diagnosis of a torsion that is caudal to the cervix; the torsion will cause a twisting of vaginal tissues that is apparent during manual palpation.

Correction of a torsion can be accomplished by rolling the animal, transvaginal manipulation, or laparotomy.[2] Correction for the majority of uterine torsions can be performed with a rolling technique described for horses, except that a plank is not required. Sedation of the female with xylazine at a dose of 0.1 mg/kg intravenously will result in lateral recumbency in most females. If the torsion is clockwise, or to the right, the animal is placed in right lateral recumbency. If the torsion is counterclockwise, or to the left, the animal is placed in left lateral recumbency. One person is needed to flex the rear legs, another is needed to control the front legs and head, and a third person is needed to stabilize the fetus. The female is rolled while the fetus is held stationary. If the torsion is greater than 180 degrees, a second rolling procedure may be needed. After rolling, a rectal examination is performed to ensure that the torsion has been corrected. If corrected, a vaginal examination is performed to see if cervical dilation has occurred. When the cervix is dilated, delivery of the cria may then be assisted. In the author's experience, over 50% of cases of uterine torsion occur at full term and delivery is assisted after correction of the torsion. If there is no cervical dilation, the female is allowed to recover and is monitored closely for recurrence.

If the cervix is open at the time of initial examination, grasping the fetus and rotating it into the correct position can be accomplished. Laparotomy is indicated in longstanding torsions or if one suspects that the uterine wall is compromised. Decisions must be made at the time of surgery if a cesarean section or simple correction of the torsion will be done. Gestation length, fetal size, uterine wall integrity, duration of clinical signs, and presence of colostrum are all factors to consider in this decision.

Multiple episodes of uterine torsion can occur in the same pregnancy and some females have a torsion in subsequent pregnancies.[2] Multiple torsions in a late-term female may necessitate a cesarean section. The prognosis is good for the dam and cria if the torsion and subsequent correction are performed in a timely manner. Future reproductive ability is generally good if the diagnosis and correction are achieved promptly.

Clinical Approach to Dystocia

When dystocia is suspected and intervention is needed, preparation and planning are important. Even though camelids seldom need sedation for examination and manipulations, adequate restraint facilities are required. Animals should be haltered and placed where restraint is adequate. Many animals will lie down when manipulations begin, so be sure that ample space is available to perform the manipulations if this occurs. Many times,

securing the animal to the corner of a stall or pen is sufficient. Placing the animal in a narrow restraint chute with immovable sides is not advised for obstetric manipulations as many females will lie down and make further treatment much more difficult. Tail wrapping is recommended; the tail can be held by an assistant or tied forward. Cleanliness of both the dam and the veterinarian is important. The dam should be thoroughly cleansed as well as the hands and arms of the obstetrician. Adequate lubrication should be used when starting the examination. Damage to the cervix is one of the major causes of infertility subsequent to a dystocia in multiparous females.

A thorough examination should reveal the nature of the dystocia and general principles of mutation and traction used in other species will allow for correction and subsequent delivery of the fetus. In rare instances dilation of the vagina may be incomplete, and a few minutes spent with gentle massage to dilate the vagina before attempting delivery is time well spent. Many times repulsing the fetus cranially will allow sufficient space to reposition an abnormal posture. Most common abnormalities encountered are one leg flexed or both legs flexed. The most difficult corrections involve a ventral or lateral deviation of the head and neck. If there is not adequate lubrication, correction can be very difficult. Many of these cases will require the obstetrician to insert both arms to simultaneously repel the cria and manipulate the neck dorsally and the nose ventrally to extend the neck.

If the presentation is breech, it is important to have adequate dilation of the birth canal before delivery is attempted. Owing to the comparative small size of the fetus and ease of repelling the cria cranially, extending the rear legs is usually accomplished with relative ease. Again, adequate lubrication is important. Placing lubrication into the birth canal and around the cria will greatly assist the delivery process.

A prolapsed uterus is relatively uncommon, but there is a higher incidence after dystocia. Owners should be warned to monitor these animals closely for several hours after an assisted delivery. If a prolapse occurs, owners should be advised to keep the animal calm and the uterus as clean as possible until treatment can be instituted. Treatment of a prolapsed uterus will require caudal epidural anesthesia and light sedation. Extending the dam's rear legs caudally will aid in replacing the uterus. Cleansing the exposed uterus as thoroughly as possible and placing the female on systemic antibiotics after repositioning the uterus will help ensure continued fertility. Administration of oxytocin and systemic antibiotics are indicated. In the author's experience, owners can easily confuse a retained placenta for a uterine prolapse. It is important to question the owner carefully if either is suspected.

Birthing Trauma and Metritis

Cervical trauma at the time of birthing is a major cause of infertility in llamas and alpacas. Care must be taken when performing any obstetric manipulations to avoid damage to the cervix. A complete vaginal examination should be done after prolonged dystocia or extensive fetal manipulations to check for damage. Postpartum metritis can occur as a complication of prolonged dystocia, contaminated birthing area, or retained placenta.[4] Clinical signs include a vaginal discharge and occasionally systemic illness. Uterine lavage and systemic antibiotics are indicated.

POSTPARTUM CARE OF THE NEONATE

A thorough examination of the cria shortly after birth is important. Alpacas and llamas have a high rate of congenital defects that range from minor to life-threatening.[3] Reported defects include choanal atresia, atresia ani, wry face, heart murmurs, polydactyly, syndactyly, cataracts, cleft palate, and umbilical hernia. If still present, removal of the epidermal membrane can be accomplished with gentle rubbing.[4] Meconium staining of the epidermal membrane can be an indicator of fetal stress.

Crias should attempt to stand within 15 to 30 minutes of birth. There should be no respiratory distress and the cria should attempt to nurse within 2 hours. Normal birth weights will range from 9 to 18 kg for llamas and 5.5 to 10 kg for alpacas. Weak flexor tendons, ears that are not erect, and teeth that have not yet erupted are all signs of a premature or dysmature cria. Initial treatments should include treatment of the umbilicus with 7% tincture of iodine or a 0.5% chlorhexidine solution. This should be repeated two to three times in the first 24 hours, especially if the cria is born in a contaminated area. Clients should be instructed to weigh crias and record the weight. Weight gain and activity are the primary signs that clients can use to monitor the health of the cria. Expect that most crias will lose weight in the first 24 hours and then start gaining. It is not unusual for alpaca crias to lose 0.12 to 0.25 kg and llamas to lose 0.25 to 0.50 kg in the first 24 hours of life. After 24 hours, crias should gain the aforementioned weights daily. Most crias will double their birth weight by 1 month of age. Body temperature of the cria should be 37.7° to 38.9°C (100–102°F). The heart rate varies between 60 and 90 beats per minute and the respiratory rate ranges from 10 to 30 breaths per minute.[5] Nursing frequency will vary with the cria's age. Newborns suckle frequently (hourly) but for a very short time. This frequency decreases with age. If the cria is born in a selenium-deficient area, 0.5 to 1 mg of injectable selenium may be given.

Failure of Passive Transfer

Early and frequent nursing is desired, because no passage of antibodies occurs across the placenta. Causes of failure of passive transfer (FPT) include lack of intake, poor production by the dam, and failure of absorption from the cria's intestinal tract. Risk factors for FPT include dystocia, premature or dysmature crias, primiparous females, and females with a history of previous FPT. Immunoglobulin levels should be determined at 24 to 36 hours of age using llama-specific radioimmunodiffusion (RID) assay kits. These kits are available from two commercial sources (Triple J Farms, Redmond WA; Bethyl Labs, Montgomery, TX). The commercially available RID plates vary in their reading[6]; using the Triple J plates, levels greater

Table **122-1**

Interpretation of Assays for Measurement of Passive Transfer of Antibodies in Neonatal Llamas

	IMMUNOGLOBULIN CONCENTRATION (mg/dl)	
Classification	**Triple J Farms**	**Bethyl Laboratory**
Complete failure of passive transfer	<400	<200
Suspect	400–1000	200–500
Adequate IgG concentration	>1000	>500

Table **122-2**

Comparative Milk Composition in the Llama, Goat, Sheep, and Cow

Animal	Water (%)	Protein (%)	Fat (%)	Energy (kcal/L)
Llama[16]	86.2	4.3	5.7	822
Llama[17]		3.4	2.7	
Goat	87.0	3.3	4.0	680
Sheep	81.0	6.2	7.9	1138
Cow	87.3	3.3	3.6	653

Data taken from Morin DE, Hurley WL, Rowan LL, Braselton WE: The composition of llama milk. VIth Congress of the ISACB, Guelph, CA, August 1994, poster 5–23.

than 800 mg/dl are considered adequate and levels less than 400 mg/dl would indicate FPT (Table 122-1). The kits are specific for llama IgG and should not be used if the cria has been supplemented with colostrum of cow, goat, or some other species. If cow or goat colostrum has been supplemented, a sodium sulfite turbidity test can be used (VMRD, Pullman WA). Measurement of total serum proteins can also be done, with the results expected to be greater than 5 g/dl. If this test is used, it is important to evaluate the total protein level and the hydration status of the cria. Dehydration and the resulting high protein level can lead to a false sense of security. It is important to note that test kits used in the equine species are not valid for assessing passive transfer in llamas and alpacas. When using the turbidity test kits, 800 mg/dl is considered adequate passive transfer for camelids.

If FPT has occurred, plasma transfusions can be given either intravenously or intraperitoneally. Llama plasma is commercially available (Triple J Farms, Redmond, WA) or can be obtained from other animals on the farm. If crias are active and not already on IV fluids, introduction of a catheter into the peritoneal cavity is a rapid and relatively stress-free procedure. A small area on the ventral midline is scrubbed and draped. A local anesthetic can be used to desensitize the area. Warmed plasma (350 to 500 ml) can be given over a period of 10 to 15 minutes. The plasma should have a concentration of at least 2500 mg/dl of IgG as measured by the Triple J plate. If an IV catheter is in place, the plasma should be infused over a 2- to 4-hour period.

If the cria is weak at birth or does not nurse vigorously or if the dam has had a history of FPT, supplemental feeding of colostrum is advised. Maintaining a bank of llama colostrum is not always feasible. Cow or goat colostrum is an acceptable substitute. Follow guidelines for other ruminant species when obtaining and handling colostrum. Advise clients that it is ideal to obtain their colostrum from multiparous females and that the first milk after birthing will generally have the highest levels of antibodies. If frozen, the colostrum should be thawed slowly in a warm water bath and not in a microwave oven. When crias are too weak to suckle or if the dam has little or no colostrum, it is advisable to feed at least 10% of the cria's body weight in the first 24 hours. Although there will be little absorption of antibodies from the gut after 24 hours, feeding colostrum, if available, for the first 3 days is advised. Supplemental feeding of the cria should be frequent and in small volumes (e.g., for an 11-kg cria, at least 8 feedings of 150 ml in the first 24 hours).

After the first 1 to 3 days of colostrum supplementation, several options are available if continued supplemental feeding is needed. Whole cow milk supplemented with yogurt (for additional calories) is a good choice. Adding 15 ml of plain yogurt to each 240 ml of milk will provide additional calories without greatly increasing the volume. Various milk replacers have also been used as long-term supplements. Goat milk or goat milk replacer appears to be the best choice for long-term supplementation (Table 122-2).

Choanal Atresia

Choanal atresia is a congenital defect characterized by either a membranous or osseous separation of the nasal and pharyngeal cavities at the level of the choanae.[5] This separation may be unilateral, bilateral, partial, or complete. As obligate nasal breathers, this condition is life-threatening and prevents the cria from suckling and breathing at the same time. Clinical signs are open-mouth breathing with marked flaring of the nostrils; there will be marked dyspnea with complete bilateral choanal atresia. Signs will be less marked if there is a partial or unilateral atresia. Airflow through the nostrils may be tested by placing a dry glass slide under the nostril to check for fogging. Conclusive diagnosis can be done by placing contrast medium in the nasal cavity and tilting the head caudally. Radiographs will demonstrate that the contrast medium will still be present in nasal cavity after 2 to 5 minutes. This will also help differentiate bilateral versus unilateral choanal atresia. Various corrective surgical procedures have been described, but all have high complication rates.[5] Many animals can survive with partial or unilateral choanal atresia, but most cases are complete and bilateral. Euthanasia for humane reasons is recommended in case of the latter.

POSTPARTUM CARE OF THE DAM

Examination of the placenta should be done to ensure that it is totally delivered. The color should be a homogeneous reddish brown. Check the portion of the placenta corresponding to the distal ends of the horns to ensure that the placenta is complete. Postpartum vulvar swelling can be marked, particularly in primiparous females. This swelling normally dissipates within 24 hours. Examine the vaginal tract for excessive bruising or tearing. Hemorrhage should be minimal after delivery of the placenta. Check the mammary glands to ensure that colostrum is present. Agalactia is uncommon in llamas and alpacas, but milk letdown may not occur until the placenta has been delivered. Many primiparous females will be reluctant to allow the cria to suckle until the placenta has been delivered. Light sedation with 5 mg butorphanol IM in alpacas or 10 mg butorphanol IM in llamas will generally facilitate the nursing and bonding process. If the primiparous female is reluctant to allow the cria to suckle, milking 60 to 90 ml of colostrum and feeding the cria with a bottle can be very helpful. Gently wash the udder with warm water and remove waxy plugs if present.

Lactation places peak nutritional demands on llamas and alpacas and most females will lose weight during the lactation period. Protein and energy levels in the feed should be increased over maintenance levels in the last 2 months of pregnancy and during lactation (Table 122-3).

Mastitis

Reports of mastitis are rare in llamas.[7] Clinical signs include reluctance to allow the cria to nurse, swollen and warm udder, and failure of growth by the cria. Milk from affected quarters may be pinkish in color and may be abnormal in consistency. Cultures should be taken and the female should be treated with the appropriate antibiotics. Intramammary infusions are difficult because of the small openings in each teat. Supplemental feeding of the cria may be needed until the infection is resolved.

Table **122-3**

Nutrient Recommendations for Late-Term Pregnancy and Lactation for Alpacas and Llamas

Nutrient	Llama	Alpaca
Protein (%)	12	15
Total digestible nutrients (%)	55–60	60–65
Crude fiber (%)	25	25
Calcium (%)	0.8–1.0	0.8–1.0
Phosphorus (%)	0.4–0.6	0.4–0.6

Adapted from Long P: *J Camel Pract Res* 1999, vol 6.

Agalactia

Agalactia may be caused by systemic diseases, mammary diseases, nutritional deficiencies, or feed toxins.[8] Agalactia in primiparous females immediately after birth may be temporary and resolve within a few hours. It is not uncommon to see milk production begin a few hours after stage three labor is complete. In such cases, oxytocin treatment may help induce milk letdown. It is speculated that females that are overconditioned during their prepubertal life may have large amounts of fat in the mammary tissue and thus have inadequate milk production. Although not proved, many speculate that there is a heritable basis for poor milk production. As in most other species, most primiparous females will have less milk production than multiparous females. Supplemental feeding of crias will be needed if there is inadequate weight gain.

Various herbal remedies are available to stimulate milk production (Llama and alpaca herbal lactation stimulator, Valley of the Llama Ranch, Sonora, CA). The author has used these stimulators with some success. Certain types of grasses contain a mycotoxin that can decrease milk production. This should show up as a herd problem, not just an individual animal problem, but small farms may only have one or two crias per season. When purchasing hay or grass seed to plant in pastures, advise owners to ask for "endophyte-free" seed. When in doubt, many forage labs can assess hay samples for endophyte levels.

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CHAPTER 123

Embryo Technologies in South American Camelids

MARCELO H. RATTO and GREGG P. ADAMS

The application of reproductive biotechnology is in the formative stages for the domestic species of South American camelids (llama and alpaca) and is virtually nonexistent for the wild species (guanaco and vicuña). Llamas and alpacas represent a valuable economic and biologic resource for Peruvian, Bolivian, and Chilean people living in the high plains of the Andes,[1] and have become a consistent feature of North American livestock production. World production of fiber from alpacas exceeds 4 million kilograms, worth more than $12 million ($US).[2] Peru has over 3 million alpacas and 1 million llamas, Bolivia has more llamas (2 million) than alpacas (325,000), and Chile has the smallest number of animals (33,000 alpacas and 67,000 llamas). There are more than 150,000 registered llamas and alpacas in North America.

Embryo transfer technologies and superstimulatory treatments have been studied intensively in other domestic species. At present, these methods are applied on a commercial basis in cattle and sheep industries to accelerate genetic improvement of the herds. Although there has been much interest, particularly from owners of valuable breeding stock in North America, little has been published on embryo transfer in South American camelids. Relatively poor results have tempered the initial enthusiasm for embryo transfer in llamas and alpacas in the early 1990s. Reasons for limited success in the use of embryo technologies in llamas and alpacas are the lack of effective superstimulatory treatments, low embryo recovery, and scanty knowledge of embryo physiology.

An overview of the current state of embryo technology in llamas and alpacas is presented herein. Ovarian superstimulation, embryo collection, and embryo transfer will be discussed as well as oocyte collection, in vitro fertilization, and embryo cryopreservation.

OVARIAN SUPERSTIMULATION

Results of studies on ovarian superstimulation and embryo production in llamas and alpacas are summarized in Table 123-1. Superstimulation has been attempted using equine chorionic gonadotropin (eCG) and follicle-stimulating hormone (FSH) during a luteal phase (induced by eliciting ovulation or by progestogen treatment) or during the sexually receptive phase. After superstimulatory treatment, the females were mated and given gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) to induce ovulation. Superstimulatory treatment schemes may be summarized as follows:

1. **Luteal phase induced by ovulation.**[3] GnRH or hCG was given when a follicle of at least 9 mm was present (day 0). At day 7, 1000 IU of eCG was administered intramuscularly. At day 9, a luteolytic dose of prostaglandin was given, and finally, to induce ovulation, 750 IU of hCG was given when follicles reached a diameter of 9 to 13 mm.
2. **Luteal phase simulated by progestogen treatment.**[4–6] The luteal phase has been simulated either by the application of a controlled intravaginal drug-releasing (CIDR) device, norgestomet, or daily progesterone treatment for 7 to 12 days. Gonadotropin treatments consisted of 20 mg pFSH (NIH-FSH-P1) IM every 12 hours for 5 days (total dose of 200 mg) or 1000 IU of eCG, starting 48 hours before progestogens are removed. Finally, 750 IU of hCG or 8 μg of GnRH were administered to induce ovulation.
3. **Sexually receptive phase.**[7,8] Females that displayed continuous sexual receptivity for 5 days received 20 mg pFSH (NIH-FSH-P1) IM every 12 hours for 5 days (total dose of 200 mg). After the last injection of FSH, females were treated with 750 IU of hCG to induce ovulation.

The preoccupation with inducing a luteal phase before or during superstimulation in camelids is enigmatic, but may simply reflect an empirical bias to conventional methods used in other ruminants. The number of ovulations or corpora lutea (CL) varies widely among studies, ranging from 2 to more than 11 per animal. Much of the variation may be attributed to the variation in follicular status at the time superstimulatory treatments were initiated. Presumably, follicular dominance will suppress the superstimulatory response in llamas and alpacas, as it does in cattle, but this remains to be tested. In a recent study[9] comparing the efficacy of FSH and eCG, treatments were initiated at the time of wave emergence and both hormones effectively induced ovarian superstimulation. FSH treatment induced the growth of 18 follicles (≥6 mm) per animal, on average, and eCG induced the growth of 17.

Table **123-1**

Superovulation Schemes Used in South American Camelids

Species	No. of Animals	Physiologic Status	Hormone	Viable Embryos per Donor	Reference
Llama	6	Luteal (hCG)	eCG	2.3	Bourke et al, 1995[3]
Llama	24	Luteal (GnRH)	eCG	1.4	Bourke et al, 1995[3]
Llama	5	Luteal (CIDR)	eCG	2.0	Bourke et al, 1994[5]
Llama	17	Luteal (norgestomet)	eCG	1.3	Bourke et al, 1995[3]
Llama	4	Luteal (norgestomet)	FSH	0	Bourke et al, 1995[3]
Llama/alpaca	4/4	Luteal (progesterone)	eCG	0	Correa et al, 1994[6]
Llama/alpaca	4/4	Luteal (progesterone)	FSH	0.5	Correa et al, 1994[6]
Llama	19	Luteal (GnRH)	eCG	1.6	Bourke et al, 1995[3]
Llama	17	Luteal (norgestomet)	eCG	1.3	Bourke et al, 1995[3]
Llama	20	Sexually receptive	FSH	1.8	Correa et al., 1997[7]; Ratto et al, 1997[8]

CIDR, controlled intravaginal drug releasing; eCG, equine chorionic gonadotropin; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin.

Table **123-2**

Embryo Collection on Different Days After Breeding from Unstimulated and Superstimulated Llamas and Alpacas

Day of Collection	Species	Region Flushed	No of Ova/ Embryos	2 to 4 Cells	8 to 16 Cells	Morulae	Blastocysts	Hatched
2–3* (*n* = 226)	Alpaca	Oviduct	141	36	24	27	—	—
4–5* (*n* = 5)	Alpaca	Oviduct	5	2	2	—	—	—
6–7* (*n* = 2)	Llama	Uterus	2	—	—	—	2	—
6–7** (*n* = 12)	Alpaca	Uterus	17	—	—	6	7	—
6–7** (*n* = 16)	Llama	Uterus	12	—	—	—	8	4

*Unstimulated.
†Superstimulated.
Modified from Del Campo MR, Del Campo CH, Adams GP, et al: The application of new reproductive technologies to South American camelids. *Theriogenology* 1995;43:21–30.

EMBRYO COLLECTION AND TRANSFER

Researchers from Peru reported the first collection of zygotes from the oviducts of alpacas after spontaneous ovulation and from superovulated females by laparotomy 3 days after ovulation.[10] The flushes were done normograde, from the ovarian end of the oviduct to the uterine end; authors suggested that the muscular uterotubal junction made retrograde flush impossible.[11] Embryos have been collected on various days after breeding, using surgical or nonsurgical techniques in unstimulated alpacas and guanacos and superstimulated alpacas and llamas (Table 123-2).

The nonsurgical method of embryo collection is similar to that used in cattle and consists of the introduction of a catheter through the cervical canal and placement of the cuff just cranial to the internal cervical os. Both uterine horns are flushed simultaneously by infusing collection medium until the horns are distended and then the medium is collected by aspiration or gravity flow. The process is repeated several times until 500 to 1000 ml of medium are recovered.[4,12–15] Uterine flushing has been done on days 6.5 to 12 after mating, but embryo recovery has been frustratingly variable. Generally less than 50% of the zygotes have been recovered, based on CL counts, regardless of the method of embryo

collection.[12] The recovery of embryos by surgical flushing of the oviduct and uterus 7 days after mating in 20 llamas treated with pFSH is summarized in Table 123-3. Llamas were mated either 0 hours or 36 hours after the last pFSH treatment.[16]

Embryo Transfer

Over the past 30 years, approximately 13 crias have been born throughout the world as a result of embryo transfer techniques[12] (Table 123-4). The first birth of an alpaca using surgical collection and transfer techniques was reported in 1974.[11] Of 44 recipients, 4 became pregnant, 3 aborted, and 1 gave birth. The first llama born using nonsurgical collection and transfer was reported in a study done in North America[17] in which collection and transfer were done 7 days after GnRH treatment. In 1987, the birth of 2 alpacas was reported in Peru through the use of nonsurgical collection and transfer.[18] Six live cria were born in the United Kingdom during 1992 to 1995, from 27 embryos transferred nonsurgically to 21 synchronized recipients.[4,5] Interestingly, only recipients synchronized with GnRH became pregnant; no pregnancies resulted in those that received progestagen implants. In Chile, the birth of 1 llama cria after 2 nonsurgical embryo transfers was reported in 1994.[19] More recently, Canadian scientists reported the recovery of 23 embryos from 5 superstimulated llamas,[20] and an American study reported the recovery of 37 embryos from 47 unstimulated donors (79%), 41% of which established pregnancies after transfer to recipients.[21] The first report of successful interspecies transfer in camelids appeared in 2001 after 2 alpaca crias were born to llama recipients.[22]

Table **123-3**

Ovarian Response (Mean ± SD) and Ova/Embryo Collection Rate after Flushing the Oviduct and Uterus in Llamas Mated 0 Hours or 36 Hours after the Last FSH Administration*

End Point	Mating at 0 Hours	Mating at 36 Hours
Number of llamas	10	10
Number of corpora lutea	4.5 ± 4.2	13.8 ± 8.4
Number of follicles ≥ 8 mm	6.5 ± 5.4	7.5 ± 8.3
Embryo collection rate	27/45† (60%)	27/138† (20%)
Embryo collection from uterus	17/27 (63%)	16/27 (60%)
Embryo collection from oviduct	10/27 (37%)	11/27 (40%)
Number of blastocysts from uterus	17	16
Number of blastocysts from oviduct	3	0
Number of unfertilized oocytes	7	11

*Given every 12 hours for 5 days, total dose of 200 mg Folltropin.
†Total number of corpora lutea.
From Ratto MH: Induction of superovulation in llamas. Master's thesis, Universidad Austral de Chile, Valdivia, Chile, 1995.

Embryo Morphology

Camelid embryos collected from superovulated females 6 to 7 days after mating vary in size from 0.1 mm to 1 mm and are usually found as hatched expanded blastocysts (Fig. 123-1). From a total of 163 llama embryos and 19 alpaca embryos, the mean diameter of the embryo was 527.1 ± 168.0 μm and 534 ± 151.4 μm, respectively.[22] In addition, great variation was noted in the diameter of successive single embryos collected from the same female in successive collections.[22] About 35% of recovered embryos were small (≤450 μm in diameter), 40% were medium (451–650 μm), and 24% were large (≥651 μm). The size distribution was almost identical for alpacas.

It appears that embryos develop more rapidly in llamas and alpacas than in other species. Morulae have been recovered from llama oviducts as early as day 3 after insemination and we have recovered a blastocyst from a llama oviduct (Adams, unpublished). Trophoblast expansion ranged from a mean of 1.2 mm in diameter on day 6.5 to 7.5, to 83 mm in length on day 13 to 14. This accelerated rate of embryo development may be related to the apparent early maternal recognition of pregnancy in these species.[24,25]

Table **123-4**

Embryo Transfer Results in South American Camelids 1968–2001

Country	Year	Species	Number of Donors	Number of Recipients	Number Pregnant	Number Born
Peru	1968	Alpacas	3	3	0	0
Peru	1974	Alpacas	15	44	4	1
US	1985	Llamas	2	2	1	1
Peru	1987	Alpacas	2	3	3	2
UK	1991	Llamas	33	11	3	2
Chile	1994	Llamas	1	2	1	1
UK	1995	Llamas/guanacos	12	10	5	4
US	2000	Llamas	47		15	
US	2001	Alpacas/llamas				2

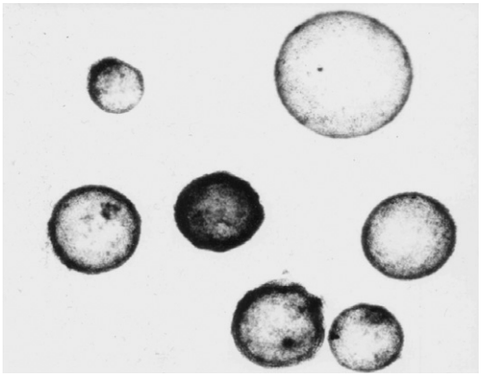

Fig. 123-1 Expanded hatched blastocysts recovered 7 days after mating by nonsurgical uterine flushing of one llama after superovulatory treatment. (From Ratto MH, Gatica R, Correa J: Timing of mating and ovarian response in llamas *(Lama glama)* treated with pFSH. *Anim Reprod Sci* 1997;48:325–330.)

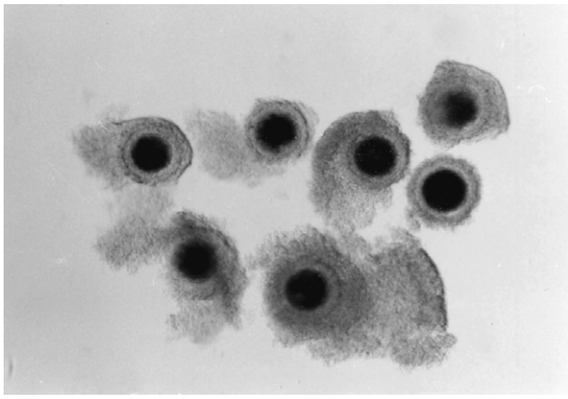

Fig. 123-2 Immature compact cumulus oocyte complexes recovered from excised ovaries of llamas by follicle aspiration using a 21-gauge needle. Note that the ooplasm is very dark, presumably due to high lipid content. (From Ratto MH, Berland M, Gomez C, et al: In vitro maturation of llama oocytes. II World Congress of South American Camelids, Cusco, Peru, 1999, p 80.)

Oocyte Collection

Oocyte recovery rate after follicular aspiration by laparotomy in 4 alpacas treated with FSH and 7 alpacas treated with eCG was 83% (105/127) and 82% (163/198) of the total follicles aspirated, respectively.[26] The proportion of expanded cumulus oocyte complex (COC) that had reached the second metaphase in the two groups was 18/45 (40%) and 16/61 (26.2%), respectively. Ultrasound-guided follicle aspiration has become commonplace as a repeatable procedure for oocyte collection in cattle, but the first report of this technique for any species of camelid appeared in a recent study in llamas.[27] The technique was used in another recent study in llamas (n = 40) to ablate all follicles 5 mm and larger in order to induce new wave emergence before superstimulation, and to aspirate oocytes from the ovaries after superstimulation.[28] An average of 15 follicles per animal were aspirated and an average of 11 oocytes per animal were collected (73% collection rate). Interestingly, administration of luteinizing hormone (LH) before oocyte collection permitted the recovery of a preponderance (~80%) of expanded COC in metaphase II; presumably these COC may be used directly for in vitro fertilization (i.e., in vitro maturation not required).

In Vitro Fertilization

In a morphologic study of the mature ovarian follicle and its contained COC,[29] growing follicles were spherical and 85% of their surface protruded from the surface of the ovary. Because llama and alpaca COCs were dark in color, they were easily distinguished through the follicle wall during transillumination,[29] and the attached COC in lamas was found to be located in the hemisphere containing the expected ovulation point. Oocytes collected from follicles 2 to 11 mm in diameter ranged from 172 to 200 µ (mean ± SD, 183 ± 14; Fig. 123-2). Immature oocytes had a very distinct and large germinal vesicle with a dark nucleolus; the metaphase plate was surrounded by a dark area easily located at 20× to 40× magnification. A greater number of oocytes was collected by mincing the ovary with a razor blade (average 27 oocytes/llama) than by aspiration of follicles between 1 and 6 mm in diameter (6.4 oocytes/llama).[30,31]

The first successful in vitro maturation and in vitro fertilization in llamas was reported in 1992 and 1994, respectively, using ovaries collected from a slaughterhouse.[30,31] Authors reported that 62% of oocytes achieved metaphase II after 36 hours of culture, and 57% of the matured oocytes displayed signs of fertilization after in vitro culture with epididymal sperm. Of 234 oocytes placed in llama oviductal epithelial cell (LLOEC) coculture for 9 days, 32% cleaved, 5.6% reached the morula stage, 6% reached the early to expanded blastocyst stage, and 4.7% reached the hatched blastocyst stage. In a more recent study of in vitro maturation using slaughterhouse ovaries,[28] maturation was complete by 28 hours; that is, there was no difference in the proportion of oocytes that progressed to the second metaphase after 28, 30, or 36 hours of culture in vitro.

Embryo Cryopreservation

The effects of two cryoprotectants, propylene glycol and ethylene glycol, on post-thaw reexpansion and morphology of blastocysts have been compared.[20] After 12 hours of culture only blastocysts preserved in ethylene glycol reexpanded, and although transfers were not attempted, authors concluded that ethylene glycol may be the cryoprotectant of choice in this species. Recently, vitrification of llama embryos has been attempted using an open pull straw (OPS) method.[32] Embryos ranging from 0.3 to 0.8 mm were exposed in one step to a high concentration of cryoprotectant (40% ethylene glycol) and then submerged directly into liquid nitrogen. In vitro reexpansion

of embryos after thawing was acceptable, but no pregnancies resulted after transfer of embryos into two recipients. High lipid content in the cytoplasm of camelid oocytes and embryos may contribute to low survival after cryopreservation.[27,32]

CONCLUSION

Traditional approaches of superovulation and embryo transfer in South American camelids have resulted in limited success primarily because of a poor understanding of reproductive physiology, puberty, and seasonality in these species. Although progress is being made, the inconsistency of reported information on the male and the limited success of semen collection and cryopreservation remain major impediments to the use of artificial insemination and embryo transfer techniques. Recent successes in embryo transfer demonstrate the promise of this technology. Interspecies transfer may be critically important for propagating threatened wild species (guanaco and vicuna). Breakthroughs in techniques for in vitro embryo production in llamas and alpacas are imminent and present many opportunities for genetic improvement of domestic herds and preserving endangered populations.

There is still much to be learned before these technologies are commonplace in camelid breeding. Little or nothing is known about camelid diseases and the potential for disease control (or transmission) with semen and embryos. To our knowledge, no cria have been produced after transfer of cryopreserved embryos or oocytes, and although preliminary data suggest that ethylene glycol may be an effective cryoprotectant, no other data are available as yet. This will provide a very important first step for assisted reproduction in exotic and endangered species and provides opportunities for movement of gametes between countries around the world.

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CHAPTER 124

Surgery of the Reproductive Tract in Lamoids

AHMED TIBARY

Surgery of the reproductive tract in camelids includes most of the elective procedures as well as therapeutic surgical techniques considered for other domestic species. As the practice of theriogenology in lamoids has developed in recent years more information has become available on the specific considerations in reproductive surgeries in llamas and alpacas pertaining to anesthesia, surgical approach choices, and pre- and postoperative management. The objective of this chapter is to review the most important reproductive surgeries in lamoids, including laparoscopic techniques.

GENERAL CONSIDERATIONS

As for any surgery, complete physical examination and assessment of the patient should be performed whenever it is possible and is a necessity in compromised patients. This assessment should include at least determination of packed cell volume, total protein, complete cell count (CBC), and fibrinogen. Serum biochemistry may be indicated in some patients (geriatric, depressed, or severely compromised patients), which may require fluid therapy. A jugular vein catheter should be placed in any compromised animal. Elective surgeries should be deferred if the animal shows clinical signs of illness. An accurate estimation of body weight is very important for calculation of medication (sedatives and anesthetics) dosage.

For minor elective surgeries, fasting for 12 hours and water deprivation for 6 to 8 hours is recommended before anesthesia. For more involved surgeries such as ovariectomy or ovariohysterectomy and laparoscopic techniques, food and water should be withheld for 24 and 12 hours before surgery, respectively.

During anesthesia, the most commonly encountered problems in camelids are bradycardia, excessive salivation, bloat, regurgitation, and aspiration. In the recumbent animal the poll of the recumbent camelid should be elevated by 10 cm above the rostral portion of the oral cavity to prevent pooling of regurgitated material in the pharynx and allow the excess saliva to run down freely. Bradycardia can be managed by administration on atropine (0.02 mg/kg IV or 0.04 mg/kg IM). Excessive saliva or regurgitated material may be removed by suction. Placement of nasogastric tube may help to reduce bloating.

Presurgical antimicrobials are commonly used by several practitioners.

ANALGESIA AND ANESTHESIA

Healthy subjects are generally easy to anesthetize in the field. Problems arise when the patient is compromised. For these compromised patients injectable anesthesia may not be the most appropriate choice, and gas anesthesia in a hospital setup should be considered.

Local Anesthesia

The most common local anesthesia used in reproductive surgery is epidural anesthesia and local blocks. Although lumbosacral epidural anesthesia has been described by some authors, it is rarely used because it causes muscle weakness and presents the risk of cranial spread to T10-11. Epidural anesthesia is generally performed in the sacrococcygeal space or between the first and second coccygeal vertebrae and provides perineal analgesia without

30. Del Campo MR, Donoso MX, Del Campo CH, et al: *In vitro* maturation of llama *(Lama glama)* oocytes. Proceedings of the 12th International Congress on Animal Reproduction, 1992, pp 324–326.
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locomotion compromise. The intervertebral space is easily identified by palpation while moving the tail up and down. The skin is shaved and aseptically prepared. An 18-gauge, 1.5-inch needle is placed on dorsal midline with the bevel facing cranially and at a 60-degree angle to the tail head. The hub of the needle is filled with the solution and advanced until the solution is pulled into the epidural space.

The duration of analgesia depends on the drug used. In the case of 2% lidocaine (0.2 mg/kg with a maximum of 1 ml per 50 kg of body weight), onset of analgesia is seen 5 minutes after administration and lasts about 1 hour to $1^1/_2$ hours. Administration of xylazine (0.1 mg/kg) provides up to 3 hours of anesthesia and the association of xylazine and lidocaine provides up to 6 hours of analgesia.

Local blocks are usually performed for abdominal surgeries with lidocaine 2% HCl. It is generally recommended to dilute this drug to 1% using isotonic bicarbonate or saline. The total dose should not exceed 4.4 mg/kg body weight (1 ml of 2% lidocaine per 5 kg of BW) to prevent lidocaine toxicity.

Sedation and Tranquilization

Sedation of the patient is accomplished easily by administration of the drug of choice intramuscularly. The most commonly used sedatives in lamoids are the alpha-2 agonists xylazine and medetomidine[1] (Table 124-1). Llamas are more sensitive to these sedatives than alpacas. Analgesia and sedation are increased by combination with butorphanol. Acepromazine is occasionally used for sedation but should be avoided in compromised animals because of its hypotensive effects and lack of reversal agents.

General Anesthesia

An association that works well in the field for anesthesia lasting 20 to 30 minutes consists of a combination of xylazine (100 mg/ml), ketamine (1000 mg/10 ml), and butorphanol (10 mg/ml). This mixture is administered intramuscularly at the dose of 1 ml/50 lb in llamas and 1 ml/50 lb plus an additional milliliter for alpacas. In pregnant animals, this association can be used for induction at half dose or with xylazine (0.05–0.1 mg/lb SQ or IM) + butorphanol (0.03–0.06 mg/lb IM).

The most commonly used induction agent is ketamine. This drug has minimal effect on the cardiovascular system, does not depress reflexes, and provides some analgesia. The major problem is that it causes muscle rigidity and increased salivation. It is better used in combination with xylazine, xylazine and butorphanol, diazepam, or guaifenesin to improve muscle relaxation (see Table 124-1).

In pregnant animals (correction of uterine torsion or cesarean section), anesthesia is generally induced with propofol (3.5 mg/kg IV) and diazepam (0.5 mg/kg IV) or guaifenesin and maintained with isoflurane in oxygen.[2] These drugs have minimal effect of the neonate. Propofol is rapidly redistributed and metabolized and diazepam has minimal placental transfer. Isoflurane is rapidly eliminated, making it well suited for cesarean section in most species.

Table **124-1**

Sedatives and Injectable Anesthetics Used in Camelids

Agent	Dose	Effect	Indications
Xylazine*	0.1–0.3 mg/kg IV 0.3–0.5 mg/kg IV	Mild to profound sedation, mild to moderate muscle relaxation, mild to moderate analgesia.	Genital manipulation, perineal surgery
Medetomidine*	0.01–0.02 mg/kg IV		
Butorphanol	0.1–0.3 mg/kg IM	Analgesia 2–4 h	Castration
	0.05–0.1 mg/kg IV	30–45 min	Preanesthetic
Butorphanol	0.05–0.1 mg/kg IM	20 min	Castration, genital examination
Xylazine	0.1 mg/kg IM		
Medetomidine	60–80 µg/kg IM	15 to 20 min	Genital examination, perineal surgery
Ketamine	2 mg/kg IM		
Detomidine	0.02–0.04 mg/kg IM	15 to 20 min	Genital examination, perineal surgery
Ketamine	2–4 mg/kg IM		
Xylazine	0.2–0.3 mg/kg IV	Anesthesia 20–30 min	Castration, laparoscopy
Ketamine	2–4 mg/kg IV		
Xylazine	100 mg	IV to effect, approximately 1–2 ml/min/50 kg body weight	Induction of general anesthesia
Ketamine	500 mg		
Guaifenesin	500 ml at 5%		
Diazepam	0.2 mg/kg IM	Immobilization 20 min	Castration, laparoscopy
Ketamine	2–4mg /kg IM		
Propofol	2–6 mg/kg IV	15–20 min	Induction for cesarean section, laparotomy
Diazepam	0.2–0.5 mg/kg IV		
Diazepam	0.1–0.5 mg/kg IV		Use in sick animals

*Reversal agents (IV): tolazoline 1–2 mg/kg, yohimbine 0.12 mg/kg, atipamezole 0.1–0.15 mg/kg.

In field practice and for rapid surgeries in healthy subjects the combination ketamine/xylazine is sufficient. The combination of butorphanol (0.1 mg/kg), xylazine (0.2 mg/kg), and ketamine (1.5 mg/kg IV) provides approximately 20 minutes of useful anesthesia for surgical procedures such as castration and testicular biopsy. I prefer to administer this combination IM. Ketamine may cause profound respiratory depression.

Triple drip (ketamine/xylazine/guaifenesin) can be administered to effect for longer duration surgeries, but anesthesia time should not exceed 1 hour. Maintenance of anesthesia can be accomplished by halothane or isoflurane in oxygen.

During recovery, the patient should be placed in sternal recumbency as soon as possible following surgery. The neck and head should be supported to prevent injury. Reversal agent should be administrated if the patient is still too sedated (see Table 124-1).

SURGERY OF THE MALE REPRODUCTIVE TRACT

Surgical procedures in the male lamoids include castration and vasectomy as well as procedures to relieve urolithiasis such as urethrotomy and tube cystomy.

Castration

Castration is the most commonly performed elective surgery in camelids. As for many species castration is indicated to prevent aggressive behavior and eliminate nondesirable males from the genetic pool. Castration may also be indicated in cases of testicular diseases.

Castration of the Normal Male

Much debate is ongoing about the most appropriate age for castration of lamoids. This is due to the effect of castration before puberty on bone growth and predisposition to arthritis. Castration at early age has been shown to delay closure of the long-bone physes, resulting in tall, straight-legged geldings and a predisposition to early onset of degenerative osteoarthritis. It is recommended that male lamoids be castrated no earlier than 15 months of age for alpacas and 18 months for llamas.[3]

Precastration considerations include thorough examination for testicular descent, lesions, and scrotal and testicular adhesions as well as evaluation of the male temperament. Presurgical administration of tetanus toxoid vaccination and antimicrobial therapy is recommended. Procaine penicillin G is generally given at 22,000 U/kg before castration and daily for 3 days.

Two techniques are used for castration of the male lamoid: scrotal and prescrotal. Prescrotal technique requires more time to complete, but is more esthetic and less painful.[3,4]

Scrotal technique. This technique is performed on the male either in the standing position or in lateral recumbency.[5,6] It is the author's preference to use the standing procedures on llamas and lateral recumbency in alpacas as the latter tend to cush, which makes manipulation and surgery very difficult and presents a risk for contamination. For standing castration sedation and analgesia is obtained by using butorphanol alone with local scrotal and testicular infiltration of lidocaine[5] or by administration of a combination of butorphanol and xylazine. Some practitioners prefer administration of an epidural, but this is generally not necessary. For recumbent patient castration a combination xylazine/ketamine or xylazine/ketamine/butorphanol is indicated. Local anesthesia of the scrotum is provided by injection of 1 to 2 ml of lidocaine 2% as a line block along the raphe median.

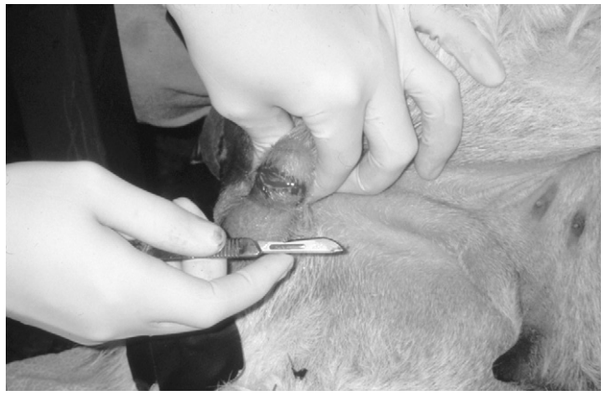

Fig. 124-1 Castration: scrotal incision.

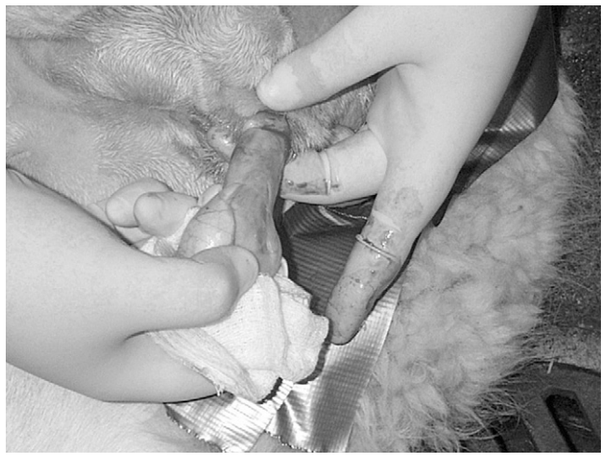

Fig. 124-2 Castration: stripping of testicular cord.

The technique is not different from that described for other species. The scrotum is prepared for aseptic surgery and an incision is made along the most ventral aspect of the scrotum by holding the testicle firmly into the scrotum to make the scrotal skin tight (Fig. 124-1). The extent of the incision is determined by the size of the testicles and is generally continued until the testicle and its envelopes protrude under pressure from the skin incision. The testicle is exteriorized and held with a towel clamp while the testicular cord is gently stripped from fat and connective tissue using a piece of gauze (Fig. 124-2). The testicle is removed using an emasculator (adult llamas) or after transfixation ligation of the spermatic cord with No. 0 chromic gut (llamas) or No. 2-0 polyglactin 910 (young llamas and alpacas) (Fig. 124-3).

Fig. 124-3 Castration: placement of transfixing suture and transection of testicular cord.

Prescrotal technique. This approach requires the animal to be in a dorsal recumbency position under general anesthesia.[4] The animal is maintained in a frog-leg position by means of ropes or straps. Strict aseptic technique is critical to prevention of infection. The skin is incised on the ventral midline immediately cranial to the ventral base of the scrotum.

In the llama a 5-cm skin incision is located 2 to 3 cm cranial to the scrotum. The left testis is moved cranially by digital pressure, and a skin incision is made directly over it. The skin and subcutaneous tissues are incised to expose the parietal vaginal tunic. The tissue surrounding the tunic is bluntly dissected to free the testis and spermatic cord, which are then retracted out of the incision. The cord is ligated proximally using No. 2 chromic gut suture material and sharply incised distal to the ligature. The contralateral testis is exteriorized through the same skin incision by manipulation into the cranial position and under the penis using digital pressure and blunt dissection and incised in the same manner. Subcutaneous fascia is closed with absorbable suture (No 3-0 polyglecaprone or No. 2-0 polydioxanone) placed in a simple continuous pattern. The skin is closed with the same absorbable suture material in a continuous subcuticular fashion.

Postoperative care for castration includes antimicrobial therapy and confinement in a small pen for 24 hours. Topical antiseptic and fly spray are indicate under farm conditions. The animal should be observed for excessive bleeding or swelling, exudative discharge due to infection, and difficulty urinating. However, postcastration complications are rare, and excessive bleeding can be managed by scrotal packing with gauze. It is important to warn the client that some males may continue displaying copulatory activity even after castration.

Castration of the Cryptorchid Male

Cryptorchidism is relatively rare in lamoids but has been reported in llamas, alpacas, and vicunas. There are very few reports on surgical removal of the retained testis. Two approaches can be utilized: parainguinal approach[7] or a laparoscopic approach (see under laparoscopic surgery).

The parainguinal cryptorchidectomy approach requires the animal to be in dorsal recumbency under general anesthesia. The inguinal area is prepared and draped for aseptic surgery. The inguinal canal is located by palpation and a 1-cm skin incision is made medial to the caudal border of the external inguinal ring and extended cranially 3 to 4 cm. The incision is continued carefully through all tissue layers into the peritoneum. The abdominal cavity is entered using two fingers and the retained testicle is identified by palpation of the area around the incision. The cryptorchid testicle lies usually just lateral to the vaginal ring. Once identified the testicle is grasped and brought up to the incision site. The spermatic cord is ligated with 2-0 polyglactin 910 and resected. The internal abdominal oblique muscle is closed with 0 polyglactin 910 in a continuous pattern. The fascia of the external abdominal oblique muscle is closed with 1 polyglactin 910 in a simple interrupted or simple continuous pattern. A subcuticular closure is provided by 2-0 polyglactin 910 in a simple continuous pattern, which apposed the skin edges. Postoperative care includes limited exercise and antimicrobial therapy for 5 days.

Vasectomy

Vasectomy is mainly used for the preparation of teasers for the induction of ovulation in camelidae for the purpose of scientific studies. However, because of its esthetic advantages some breeders may opt for this technique rather than castration to sterilize males that are undesirable for reproduction.[3]

Vasectomy can be performed in the llama in a sitting or standing position after sedation, but the dorsal recumbency is the preferred position in alpacas. The scrotal skin is prepared by clipping and surgical scrubbing. Surgical drapes are placed around the scrotum. A 2- to 4-cm vertical scrotal skin incision is made slightly medial on the cranial surface of the neck of the spermatic cord. The spermatic cord is freed by blunt dissection and exteriorized with the help of hemostatic forceps (Fig. 124-4). The vas deferens can be easily identified by palpation or visually by its white color and the presence of adjacent vein and artery. The vas deferens is exteriorized using forceps or a spay hook through a small nick made in the vaginal tunic. A 3-cm portion of the vas deferens is removed after ligating each end (Fig. 124-5). The vaginal tunic does not need to be sutured. The skin is sutured, and the same procedure is repeated on the other side. Excised tissue should be submitted for histologic confirmation.

Another approach to vasectomy is to make the incision near the inguinal canal. This technique provides an easier identification of the vas deferens, particularly in individuals that have a short testicular cord. A subcuticular suture provides closure of the surgical site. Alternatively, a 2-cm segment of the ductus deferens may be excised via standard laparoscopy using a forceps scissors[8] (see discussion of laparoscopic surgery).

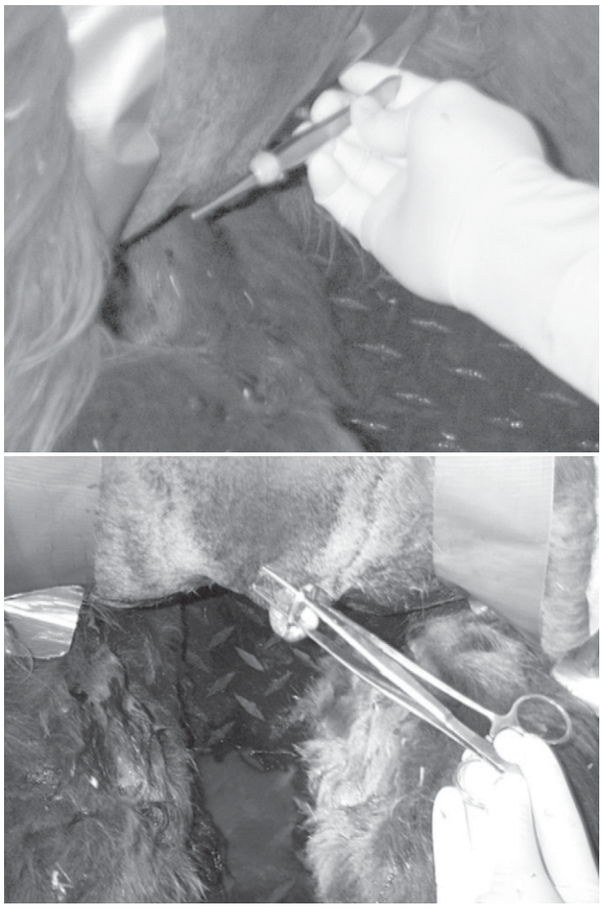

Fig. 124-4 Vasectomy: exteriorization of the testicular cord.

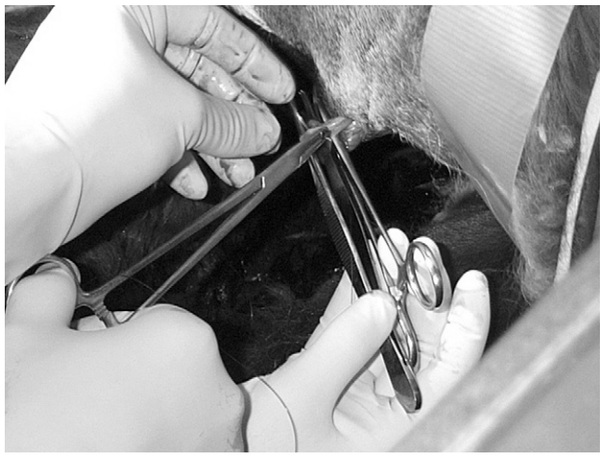

Fig. 124-5 Vasectomy: ligation and transection of vas deferens.

SURGERY OF THE FEMALE REPRODUCTIVE TRACT

Cesarean Section

Cesarean section is indicated if vaginal delivery is impossible. The most common causes of dystocia requiring cesarean section include failure of cervical dilation, uterine torsion, and fetal malpresentation. In alpacas, delivery by cesarean section may be indicated even if the fetus is dead because of the difficulty of manipulation and procedures such as fetotomy. Damage to the cervix or uterus is more likely to occur when trying to force manipulation of the fetus because of inadequate space or cervical dilation. The decision to proceed with a cesarean section should be made relatively quickly in order to improve the chances of fetal and maternal survival. If the size of the dam precludes transvaginal palpation, immediate cesarean section should be chosen.[9]

The health and degree of compromise of the parturient female and the fetus should be assessed. I recommend placing a jugular vein catheter in all females presented for dystocia. Females presenting with cardiovascular shock, dehydration, and hypotension should receive crystalloid fluids and nonsteroidal anti-inflammatory drugs. Presentation, position, and posture of the fetus may be determined by vaginal or rectal palpation. It is preferable to perform these examinations after epidural anesthesia. Viability of the fetus may be determined by transabdominal ultrasonography. However, in many cases there is no time to proceed with fetal evaluation.

Two approaches are utilized for cesarean section in lamoids: the ventral midline approach and the left paralumbar approaches.[9,10]

Ventral Midline Approach

The ventral midline approach has been suggested as the preferred approach for alpacas and llamas. This allows simple access to the abdominal cavity and complete exteriorization of the uterus with minimal hemorrhage. Anesthesia is generally induced with propofol and diazepam (0.5 mg/kg IV) or guaifenesin. Although some have used halothane, maintenance of anesthesia is usually provided by isoflurane in oxygen.[11] The patient is placed in dorsal recumbency and the ventral midline is prepared aseptically. A midline celiotomy incision (25 cm in alpacas and 35 to 40 cm in llamas) is made through the skin, subcutaneous fat, cutaneus trunci muscle, and linea alba from the cranial border of the mammary gland extending cranially (Fig. 124-6). The uterus is identified by direct palpation and exteriorized from the abdomen.[11]

An incision is made through the uterine wall over the hindlegs along the greater curvature. The cria is removed and the umbilicus clamped and transected. If the placenta is still adhered to the uterine wall, it should be left in place but peeled off along the uterine incision to provide adequate hemostasis and closure. Because of the type of placentation mural bleeding may be a problem. The margins of the uterine incision are oversewn with a continuous interlocking pattern using resorbable suture

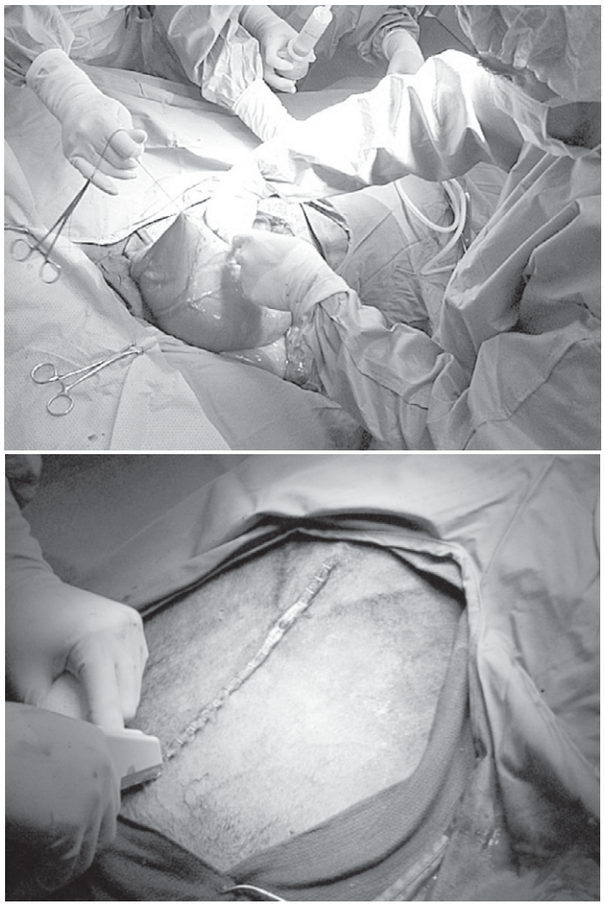

Fig. 124-6 Cesarean section: midline ventral abdominal approach; skin incision.

material (No. 1 chromic gut or smaller in alpacas) to control bleeding before closure of the uterus (Fig. 124-7). The uterine serosa is closed using No. 0 Vicryl, No. 0 polydioxanone or polyglecaprone in alpacas or No. 2 chromic gut (llamas) in a simple Cushing or Utrecht. Some practitioners prefer to administer 10 to 20 IU oxytocin in the uterine wall. The uterine wall and abdominal cavity may be lavaged with warm saline solution containing antibiotics (1 L isotonic saline solution containing antibiotics (K-penicillin G 22,000 U/kg body weight, sodium ampicillin 20 mg/kg, or sodium ceftiofur 1 mg/kg), anti-inflammatory drugs (flunixin 1 mg/kg), and heparin (20 to 40 units/kg). Carboxymethyl cellulose (CMC 14 ml/kg body weight, intraperitoneally) may be used to prevent postoperative adhesions but this has not been thoroughly evaluated in llamoids.[11,12]

The linea alba is closed by appositional pattern with interrupted horizontal mattress sutures, cruciate pattern sutures, or continuous suture pattern. In alpacas various suture materials can be used: No. 2 polyglycolic acid, No. 1 polydioxanone or polyglactin 910, No. 2 Vicryl. Closure of the skin may be done with staples, or 2 PDS horizontal mattress suture pattern, or preferably a subcuticular suture pattern (No. 2-0 polyglactin 910 or polyglecaprone); do not use skin sutures.[12]

Paralumbar Technique

The animal is sedated and restrained in a sitting sternal position. In some females only physical restraint and line or inverted "L" block anesthesia are needed. Epidural anesthesia may help restrain the animal in selected cases. The surgical site is clipped and shaved over an area extending vertically from the processes of lumbar vertebrae down and horizontally from the last rib to the hip (Fig. 124-8). The skin incision is oblique, extending from the angle formed by the hip to the bottom of the last rib. The incision line should be parallel to the direction of the thighs while the animal is sitting in the sternal position (Fig. 124-9). The muscle layers are opened by blunt dissection in a grid fashion. The peritoneum is incised and the hand of the operator is introduced into the abdominal cavity and directed caudally to the pelvic region where the uterus is identified by direct palpation. The gravid uterine horn (nearly always the left) is grasped around a fetal limb and gently exteriorized from the incision (Fig. 124-10). Uterine incision, exteriorization of the fetus, and uterine closure is conducted in the same manner described previously.[9]

The abdominal cavity is closed in three layers: (1) The peritoneum and the transverse abdominal muscle *(m. transversus abdominis)* are closed together with a simple continuous suture. (2) The internal and external abdominal muscles (*m. obliquus internus abdominis* and *m. obliquus externus abdominis*) and the subcutaneous fascia are closed in a second layer with a simple continuous suture. The suture is anchored every 2 to 3 cm to the transverse muscle to remove any dead space and prevent formation of pockets. (3) The skin is closed using a continuous interlocking suture pattern (Fig. 124-11).

Some practitioners prefer to close all muscles in one layer to provide a better support and decrease tension on each individual layer and prevent herniation. This is provided by a simple continuous suture pattern or cruciate suture pattern using No. 1 PDS or No. 1 polyglactin 910 suture.

Postoperative Care and Complications

Postoperative care includes pain management using butorphanol tartrate (0.05 mg/kg, IV) or flunixin meglumine (1 mg/kg IV, once daily) and antimicrobials in the form of benzylpenicillin (20 mg/kg IV four times daily), gentamicin sulfate (6.6 mg/kg IV once daily), or ceftiofur sodium (5 mg/kg IV twice daily). Flunixin meglumine is continued for 3 days. Antimicrobial prophylaxis should be continued for 5 to 7 days depending on the condition of the uterus and fetus at the time of surgery. Ulcer prophylaxis (e.g., omeprazole, 2 mg/kg, PO, every 12–24 hours for 5 days) is routinely administered after surgery. Fluid therapy may be indicated in some cases. The dam should be monitored for postpartum metritis and toxemia. The placenta is generally expelled within a few hours if the cervix is open or 2 to 4 days if it was closed at the time of surgery. Oxytocin administration (20 to

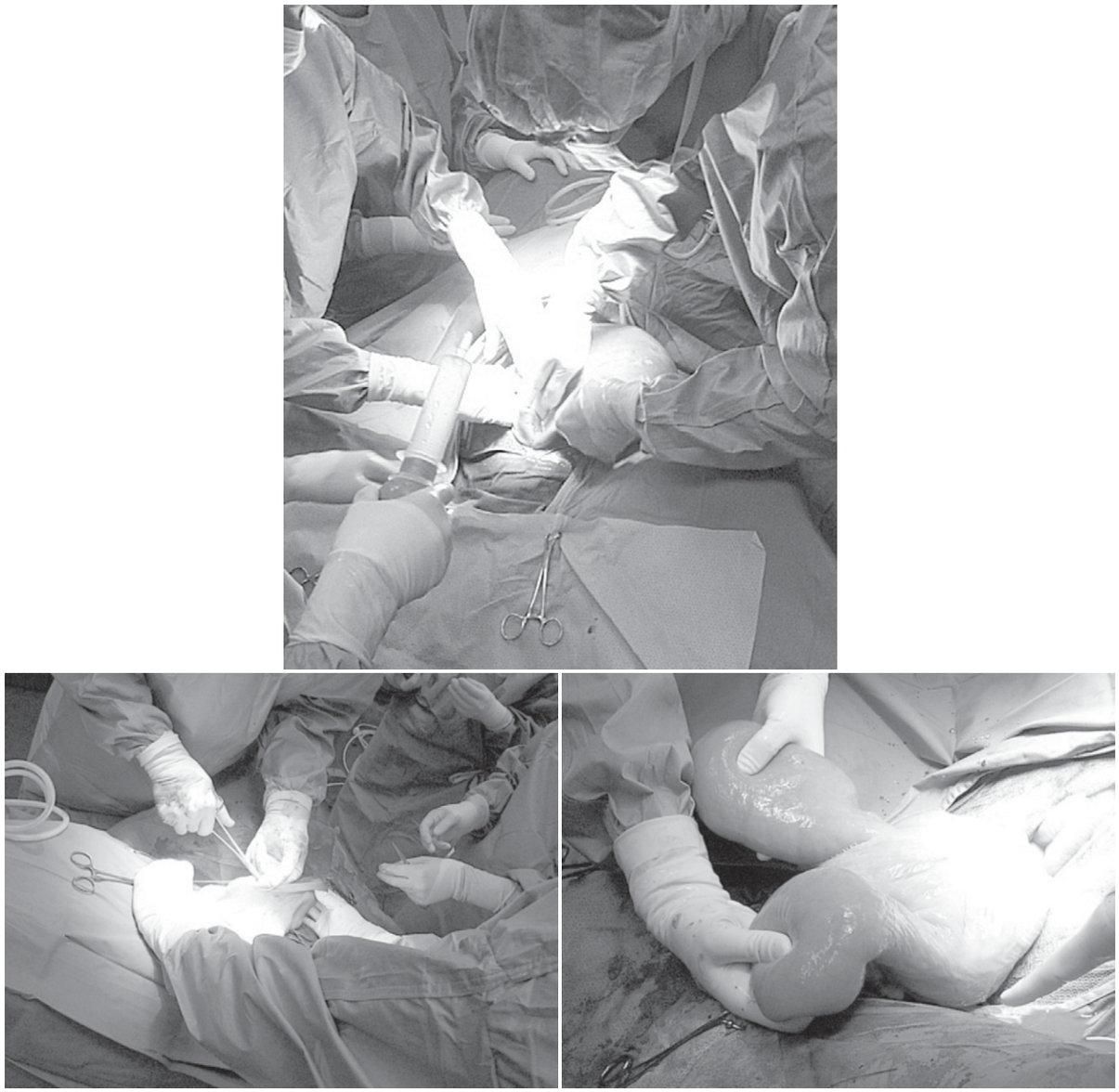

Fig. 124-7 Cesarean section: midline ventral abdominal approach; exteriorization and suture of the uterus.

30 IU, IM) every 6 hours during the first 24 hours after surgery is advocated by some practitioners.

Information regarding success rates and complications associated with cesarean section in llamas and alpacas is limited. Some of these complications include incision infection, hernia, peritonitis, intestinal adhesions, and infertility. However, these complications are very minimal when the surgery is performed early in dystocia and sterile technique is used. The rebreeding success rate is excellent, and most females will be rebred within the first 4 months after surgery.

Ovariectomy

Ovariectomy is usually performed for convenience to prevent sexual activity or eliminate pregnancy or to remove diseased organs (ovarian masses). In camelids, ovariectomy can be done via a parainguinal, ventral midline, or flank approach. The choice of a particular technique depends on the age of the animal, the side of the ovary concerned (unilateral or bilateral), and the situation of the ovary (normal versus abnormal). The flank approach can be used in multiparous animals because the

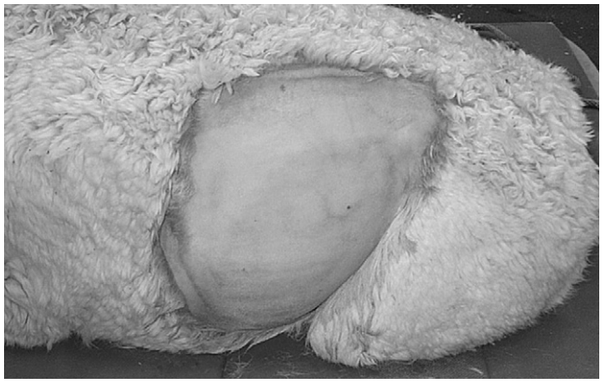

Fig. 124-8 Cesarean section: paralumbar approach; surgical site preparation.

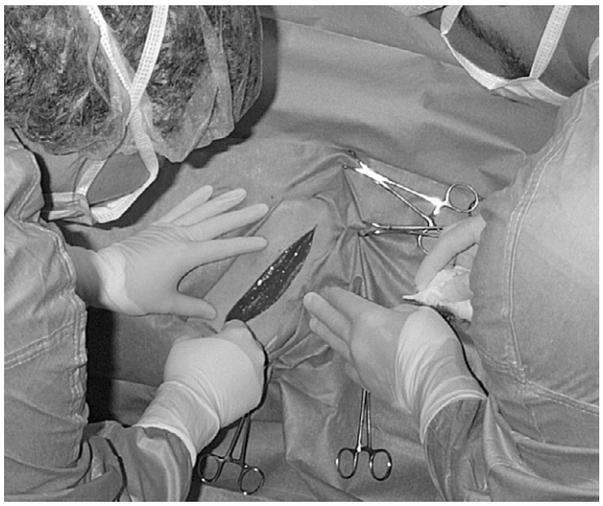

Fig. 124-9 Cesarean section: paralumbar approach; skin incision.

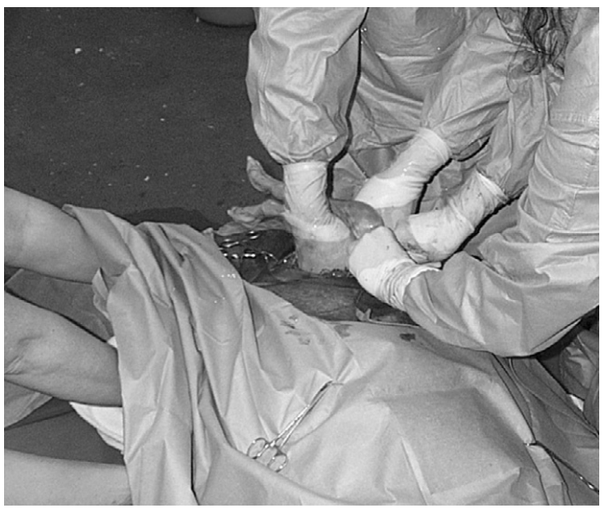

Fig. 124-10 Cesarean section: paralumbar approach; exteriorization of the uterus.

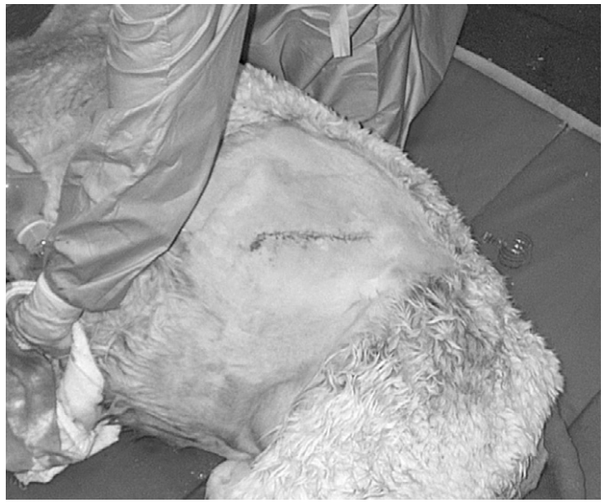

Fig. 124-11 Cesarean section: paralumbar approach; skin closure.

genital tract is sufficiently developed to allow exteriorization via the flank. In young animal ovariectomy or in presence of large ovarian masses or when both ovaries need to be removed, the ventral midline approach is the preferred technique.[9] Because the uterus is extremely contracted during the follicular phase the author has found that ovariectomy and ovariohysterectomy are best accomplished after a period of progesterone therapy to simulate a luteal phase (progesterone 50 mg IM per day for 10 days). Anesthesia and preparation of the animal for ovariectomy is similar to the technique described for cesarean section. A small incision (4 to 6 cm in alpacas and 6 to 8 cm in llamas) is made through the skin and all layers of the ventral midline starting just cranial to the udder and continuing cranially. The surgeon introduces two fingers into the abdominal cavity (Fig. 124-12). The urinary bladder is identified by palpation, and the uterus is recognized in its dorsal aspect by following one of the horns to the uterine bifurcation. One of the uterine horns is grasped between the fingers and pulled toward the surgical incision. Both horns are exteriorized by gentle traction (Fig. 124-13). The ovary is exteriorized from the incision site by applying gentle traction. The vascular pedicle (proper ligament) of the ovary is isolated by passing forceps through the mesovarium and clamped with hemostatic forceps, making sure to incorporate the ovarian artery and vein. The ovary is transected after transfixion ligation with absorbable suture material. Closure of the surgical site and postoperative care are performed as described for cesarean section.[9]

Ovariohysterectomy

Ovariohysterectomy is indicated when there is uterine pathology. The procedure is done under general anesthesia with the animal in dorsal recumbency. The animal should be fasted 24 to 48 hours and receive systemic antibiotics 12 hours before surgery. Although a parainguinal approach has been described, in the author's

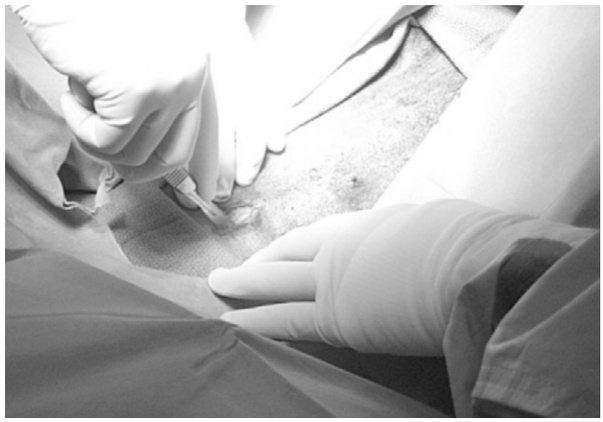

Fig. 124-12 Ovariectomy, incision site.

Fig. 124-13 Ovariectomy, exteriorization of the uterine horns and ovary.

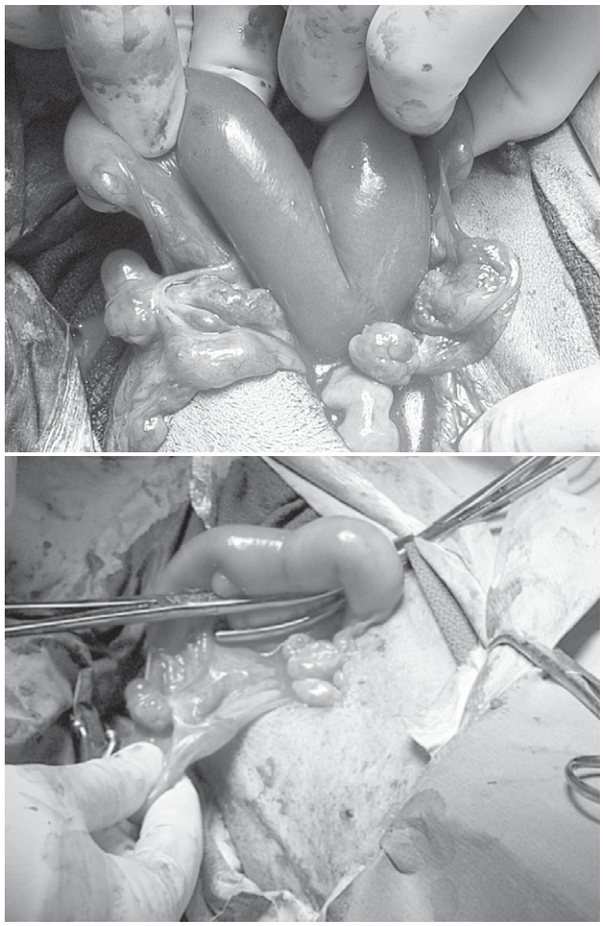

Fig. 124-14 Ovariohysterectomy: transfixion and resection of the uterus.

opinion the best surgical approach to ovariohysterectomy is the ventral midline approach. The animal is prepared as for a cesarean section. A skin incision is made from the cranial border of the mammary glands toward the umbilicus. The extent of the incision depends on the size of the uterus. The uterus is completely exteriorized from the surgical opening and all fluids present are drained with a large-bore needle attached to a flexible tube. The uterus should be flushed with saline solution and antibiotics and then sutured to avoid contamination of the abdominal cavity. All blood vessels including the ovarian and cranial uterine arteries are identified and ligated (double ligation). The broad ligament of the uterus can then be transected to allow manipulation of the uterus at the cervical level. Transfixation ligatures are placed proximal to the cervix, making sure to include the large uterine vessels located on each side. The uterus is transected at the level of the body between two hemostatic forceps (Fig. 124-14). A circumferential ligature of absorbable suture material is placed close to the cervix. If the remaining portion of the uterine body is large, it should be closed with an inverting suture pattern before replacing it in the abdomen. The vagina should be closed with overlapping mattress sutures with catgut or Vicryl. The reproductive tract is amputated proximal to the mattress sutures. The serosal surfaces of the stump are apposed by simple interrupted sutures. The abdomen is closed in the same manner as for cesarean section.[9]

Perineal Laceration Repair

Perineal lacerations are relatively uncommon but do occur in lamoids. They are usually classified using the same grading system used in the equine species.[13] The author has seen mainly third-degree and occasional second-degree perineal lacerations. The occurrence of third-degree laceration is probably due to the small perineal body, which is characteristic of this species.[13]

Superficial lacerations can be repaired quickly under local anesthesia using Caslick's procedure if the tear has already epithelialized. Second-degree laceration requires

reconstruction of the perineal body, generally after sedation and epidural anesthesia.

Repair of the third-degree perineal laceration and rectovaginal fistulae should be delayed until the tissue has granulated and epithelialized. Although some practitioners perform this operation as early as 2 weeks after injury, it is generally recommended to wait until 4 to 8 weeks post partum. Repair of third-degree laceration is preferably done on the standing sedated patient; however, the practitioner should be aware that many animals, particularly alpacas, have the tendency to go down in a sternal position during surgery. Epidural anesthesia is helpful because the rectal and vulvar tissues need to be retracted using suture or retractors. Repair techniques are similar to those described for the mare and consist of dissection of the rectal and vaginal walls with the goal of creating a new separate rectal floor and vestibular roof and reconstruction of the anus.[13]

Fig. 124-15 Truss (shoelace suture) for vaginal prolapse.

Vaginal and Uterine Prolapse

Vaginal prolapse occurs in lamoids during the last few weeks of pregnancy. Predisposing factors are age, parity, and obesity. Different degrees of vaginal prolapse may be observed, ranging from small protrusion of the vestibular area when the female is in the sitting position to permanent vaginal prolapse. Management of the condition consists of cleaning and replacement of the exposed area. The vaginal tissue is retained by truss (shoelace) pattern. This is accomplished by placing three loops of suture on each side of the vulvae and running an umbilical tape through the loops in a shoelace manner (Fig. 124-15). The manipulation may be done after epidural anesthesia or local block, depending on the extent of tissue to be replaced.

Uterine prolapse occurs in lamoids, but there are no precise data on the incidence of this problem. Most of the cases seen by the authors occurred with 2 hours of normal birth or after induction of abortion. The condition is suspected to be associated to hypocalcemia. Uterine prolapse is replaced in the same manner described for other species after epidural anesthesia. The placenta is gently peeled off the endometrium before if still present. Truss pattern is placed on the vulva to prevent recurrence of the prolapse. Prognosis for fertility depends on the health of the uterine tissue at the time of diagnosis. Complications include uterine blood vessel rupture, uterine wall laceration, and cervical laceration. Large-spectrum prophylactic antimicrobials should be administered for 5 days to prevent infectious complications. Nonsteroidal anti-inflammatory drugs may be used in the first 2 days.

Vaginal Stricture

Vaginal strictures can be congenital or acquired. Thorough evaluation of the vestibular and vaginal cavity requires sedation and epidural anesthesia. A videoendoscopy of the area may be required for completeness of evaluation. Acquired vaginal strictures are usually due to vaginal and vestibular adhesions. Congenital vaginal strictures include imperforated hymen and other serious congenital anomalies such as vaginal hypoplasia or aplasia. When evaluating vaginal strictures, it is important to keep in mind that double vagina (didelphys) may be perceived as a stricture. "Persistent" hymen may be ruptured by manual dilation or scissors. In some instance the author has used different size bougies to evaluate and expand the narrowing of the vestibular area.

Acquired vaginal adhesions may be due to traumatic lesions following dystocia. Complete vaginal occlusions are associated with pyometra or mucometra. Drainage of the uterine content can be attempted by surgical or laser removal of the adhesion. Thickness of the adhesion should be evaluated. One technique used by the author is to place a Foley catheter with the fluid-filled balloon completely against the vaginal occlusion and evaluate by transrectal ultrasonography the distance from the caudal aspect of the uterine/vaginal fluid to the wall of the balloon.

LAPAROSCOPY AND LAPAROSCOPIC SURGERY IN LAMOIDS

Laparoscopic techniques are becoming commonplace in many hospitals and referral centers for evaluation of the reproductive tract or reproductive surgeries. Laparoscopy can be performed with the animal standing or in dorsal recumbency.[14]

Standing Laparoscopy

Standing laparoscopy is performed mainly in llamas for in situ observation of the genital organs or ovariectomy. Food is withheld generally for 24 hours and water is withheld for 12 hours before surgery, respectively. Llamas are restrained in stocks and sedated with butorphanol tartrate (0.1 mg/kg BW IV). The left paralumbar fossa is prepared for aseptic surgery and 2 % lidocaine is infused into the subcutaneous and muscular tissues in an inverted "L" pattern to desensitize the region.[15]

A small (10 to 15 mm) skin incision is made in the craniodorsal portion of the fossa 4.5 cm caudal to the twelfth rib and 8 cm ventral to the transverse processes of the lumbar vertebrae. This provides a portal for a 30-degree laparoscope, which is placed after penetrating the abdominal cavity through the abdominal muscle and peritoneum using an appropriate trocar. The abdomen is distended with carbon dioxide to a pressure of 10 mm Hg. Each ovary is identified by following the respective uterine horn craniad. This manipulation may be difficult if the urinary bladder is distended. Once the genital tract has been located and inspected a second incision is made in the skin of the caudodorsal portion of the left paralumbar fossa 10 cm caudal to the twelfth rib and 10 cm ventral to the transverse process of the lumbar vertebrae. This incision will provide the portal for a manipulation instrument (grasping forceps) inserted through a cannula fitted with a sharp trocar. A small incision is then made in the skin of the caudoventral region of the left paralumbar fossa 6 cm caudal to the twelfth rib and 15 cm ventral to the transverse processes of the lumbar vertebra to provide a portal for the introduction of a ligature guide holding a loop of size 0 polydioxanone. The ovary is grasped with the forceps through the suture loop. The ligature is pushed to the level of the ovarian pedicle and tightened around it. The ligature guide is removed and scissors are introduced to cut the end of the ligature and transect the ovarian pedicle at the base of the ovary. The ovary is removed from the abdomen with the grasping forceps. All instruments are removed and excess gas expelled. The portal incisions are closed (external oblique muscle with a single cruciate 3-0 polyglyconate and skin is closed with a single cruciate suture 3-0 monofilament nylon).

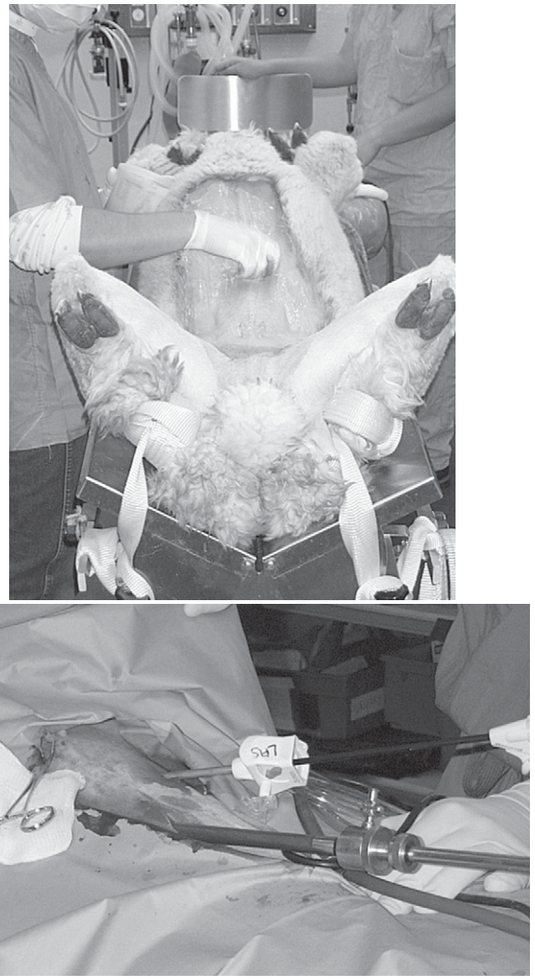

Fig. 124-16 Laparoscopy, ventral midline approach.

Ventral Abdominal Approach

This approach is very useful for examination of the reproductive tract, insemination, oocyte collection, and embryo transfer as well as ovariectomy and ovariohysterectomy.[11,14] The technique is performed under general anesthesia in a dorsal recumbency position. Quick visualization of the reproductive tract for the purpose of inspection and insemination may be done with sedation and local block.

Feed is withheld for 24 hours before surgery and antimicrobial therapy (PPG 22,000/kg IM) is given every 12 hours before and up to 72 hours after laparoscopy. Anesthesia is induced with an intravenous combination of xylazine hydrochloride (0.25 mg/kg) and ketamine hydrochloride (3.5 mg/kg) and maintained with isoflurane.

The ventral abdomen is clipped and surgically prepared from the cranial edge of the mammary gland to the xiphoid (Fig. 124-16). A small skin incision (size depending on the diameter of the laparoscope) is made in the midline at the level of the umbilicus or 3 to 5 cm caudal to it (see Fig. 124-14). The abdomen is perforated with a teat cannula and insufflated with CO_2 to a partial pressure of 10 to 20 mm of Hg. The laparoscope trocar and cannula are inserted at this level through the linea alba and peritoneum into the abdomen. The trocar is removed and replaced with a laparoscope. The pelvic inlet is identified by tilting the surgical table to 30 degrees to elevate the hind legs and displace the abdominal viscera cranially. The instrument portals are made on the left and right side of the midline midway between the scope portal and the caudal aspect of the fold of the flank.

The uterus is located under the urinary bladder and each uterine horn is followed to its tip and elevated to allow visualization of the corresponding ovary (Fig. 124-17). Atraumatic grasping forceps are used to manipulate the uterus and bladder for location of the ovaries.

For ovariectomy, the ovary is dislodged from the ovarian bursa by gentle manipulation until the mesovarium and proper ligament of the ovary are isolated. Hemostasis may be provided by two Hulka clips across the mesovarium or proper ovarian ligament or a suture loop placed around these structures. The proper ligament is incised with scissors proximal to the clips or suture.[11]

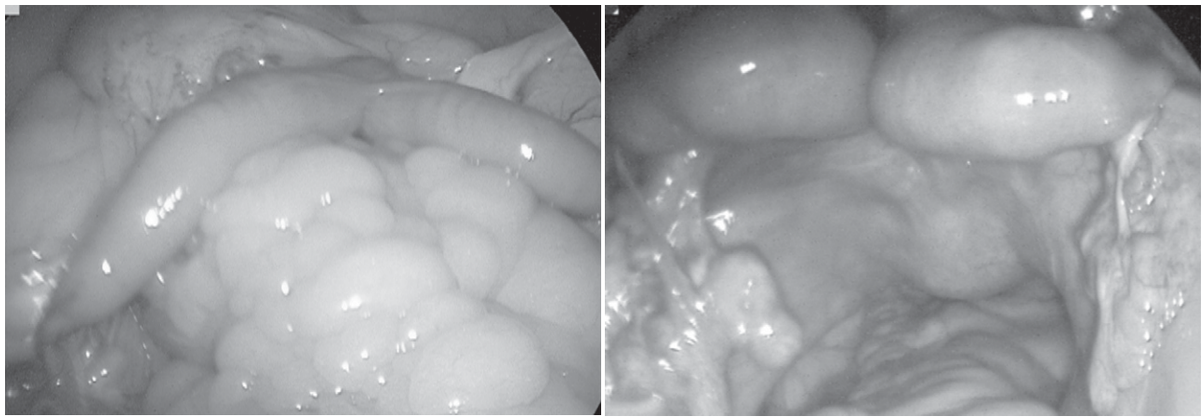

Fig. 124-17 Laparoscopy, visualization of the uterus and ovary.

Each portal, the linea alba, and the external sheath of the external abdominal muscle are closed with one simple cruciate suture of 0 polyglycolic acid and the skin is closed with 2-0 polypropylene using a simple cruciate suture.

Laparoscopic ovariohysterectomy has been described and requires good practice in the manipulation of suture and dissecting instruments through a laparoscope. The female is prepared in the same manner as for ventral midline laparoscopic ovariectomy. The procedure starts with ligation of the ovarian vessels. The mesovarium is exposed and incised after hemostasis has been ensured by a series of clips using a Ligaclip inserted into the left portal. The broad ligament is transected close to the uterine horn but avoiding the uterine artery. The broad ligament is cut caudal to the uterine body. The same procedure is repeated on the other side. Once both uterine horns are freed a ligature loop is introduced and the uterine horns and ovaries are passed through all the way to the uterine body and tightened. A second ligature loop is placed in the same manner around the uterine body to provide hemostasis. The uterus is transected after a third loop is placed along the portion of the uterus to be removed to prevent uterine content spillage. The uterus and ovaries are removed from the abdominal cavity from one of the instrument portals after increasing its size.[14]

Laparoscopic Vasectomy

Laparoscopic vasectomy has been reported in llamas and alpacas. The technique is performed on the patient in dorsal recumbency after sedation.[8] The author prefers to operate under general anesthesia. The surgical table is tilted to 45 degrees with the hindquarters elevated. Depending on the length of instrument the laparoscope portal can be at the level of the umbilical scar or more caudal while the manipulation and incision instrument portals are placed on each side of the penis about 5 cm to the rudimentary teats. The incisions for the instrument portals may be made in such a way that the skin incision is not parallel to the abdominal incision.

The ductus deferens is located at the site where it emerges through the inguinal ring where it joins the contralateral ductus deferens dorsal to the urinary bladder and 2 to 3 cm of the ductus deferens are resected between these sites.

Portal incisions are closed with simple interrupted pattern. Examination of ejaculate at 15 days and 30 days showed presence of sperm cells but no motility; after 45 days no sperm cells were found.

Laparoscopic Cryptorchidectomy

Laparoscopic cryptorchidectomy is modified from a technique described in stallions. Feed is withheld for 48 hours before surgery. Antibiotic preventive therapy consisting of IM injection of procaine penicillin G (22,000 IU/kg every 12 hours) was started 2 hours before surgery and continued for 72 hours. Anesthesia is induced with a mixture of ketamine hydrochloride/xylazine/butorphanol and maintained with isoflurane in oxygen. The patient is positioned in dorsal recumbency. The abdominal area from the xiphoid process to the inguinal region is clipped and aseptically prepared and draped for surgery.

A 5-mm stab incision is made at the umbilicus and a teat canula is inserted into the abdomen for insufflation with carbon dioxide (CO_2) to a pressure of 15 mm Hg. The laparoscope is inserted as described previously, and the surgical table is inclined in the head-down position to allow cranial displacement of the abdominal viscera and localization of the retained testis. Three instrument portals are used: two are located on the same side as the retained testis and one is on the opposite side and more cranial. All instrument portals are located within the axial one half of the rectus abdominis muscle and lateral to the midline. The portals are created by making a 1.5-cm incision through the skin and the external rectus sheath. Blunt penetration through the remaining abdominal wall is performed with a 5-mm conical tip obturator. Instruments are subsequently placed through these portals without using an outer cannula.

Once the testis is located and isolated with a manipulation rod, a grasping forceps is introduced through the

cranial portal, and a laparoscopic ligature is introduced the caudal portal. The laparoscopic ligature loop is made of polyglactin 910 (Vicryl), and modified Roeder knot is tied forming a loop. The long tail of the suture loop is threaded through a hollow steel push rod that serves to advance and tighten the slipknot (Fig. 124-18). The push rod is made by drilling a 1.2-mm hole in the end of a 10-gauge, 26.5 cm bitch catheter. The push rod and ligature is inserted into the caudal portal through an introducer sleeve. The grasping forceps is passed through the loop of ligature, and then the Chambers catheter is used to manipulate the testis into position for the grasping forceps. The testis is grasped near the head of the epididymis and elevated from the surrounding tissues. The ligature is then advanced around the testis and pedicle to a point just proximal to the epididymis. When the ligature is correctly positioned, the push rod is advanced while pulling the long tail of the loop to advance the knot and tighten the ligature. The testicle is divided distal to the ligature using scissors placed through the opposite portal. The suture material is cut approximately 1 cm from the knot. After transection, the ligated pedicle is observed for hemostasis. The scissors and push rod were removed from the abdomen. A pair of forceps is passed through the caudal portal, and the testis is transferred to them and removed from the abdomen. After removal of the testicle, the abdomen is decompressed and the operative table returned to a horizontal position. The laparoscopic portal is closed with polyglactin 910 in a figure 8 pattern. All skin incisions were apposed with a subcuticular, simple continuous pattern of polyglyconate, then sealed by application of cryoacrylate, taking care not to interpose the glue between wound edges. Skin adhesive is applied around the incision sites to facilitate application of elastic bandage tape.

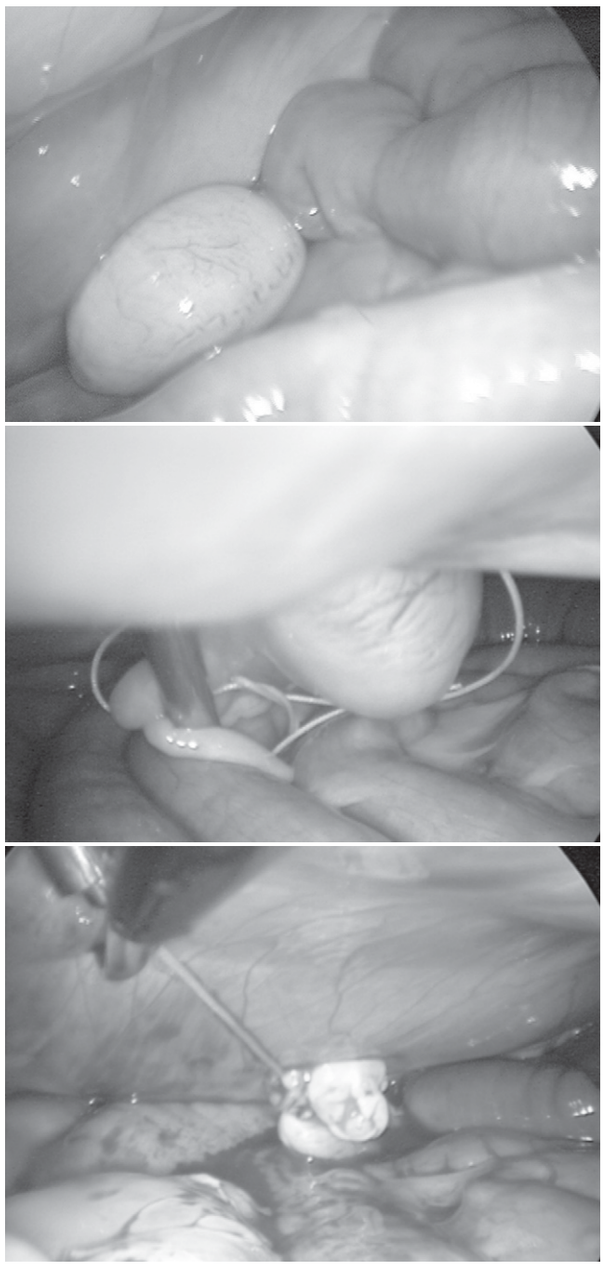

Fig. 124-18 Laparoscopic cryptorchidectomy.

SURGERY FOR SPECIAL REPRODUCTIVE PROCEDURES

The surgical techniques described so far are standard technique to access any part of the cranial reproductive tract in the female Camelidae and can be used for some special reproductive procedures such as direct transfer of embryos in the uterine lumen (surgical embryo transfer), collection of early stages of development of embryo from the uterine tube, gamete intrafallopian transfer (GIFT), and follicular aspiration for oocyte collection, although this latter technique is quickly being replaced by ultrasound-guided transvaginal aspiration.

Collection of oviductal stage of embryo development and GIFT are easily accomplished when the ovary, ovarian bursa, and uterine tube are exteriorized. Transfer of gametes is readily accomplished by injecting them directly into the uterine tube after catheterization. The oviduct is catheterized by placing a plastic catheter from the infundibular end and maintaining it in place with forceps. Oviductal flushing may be used to evaluate uterine tube patency in females with longstanding infertility.

Embryo collection is generally done under general anesthesia. An area similar to that described for ovariectomy or ovariohysterectomy is prepared for surgery and draped. The uterus and ovaries are located and exteriorized as described earlier. The ovaries are inspected for numbers of corpora lutea (Fig. 124-19). During the procedure, the uterine horn should not be allowed to dry out. This is accomplished by lavage with sterile saline. Each horn is flushed separately. A small incision is made at the base of the horn, and a Foley or Argyle silicon No. 10 catheter is introduced and maintained in place

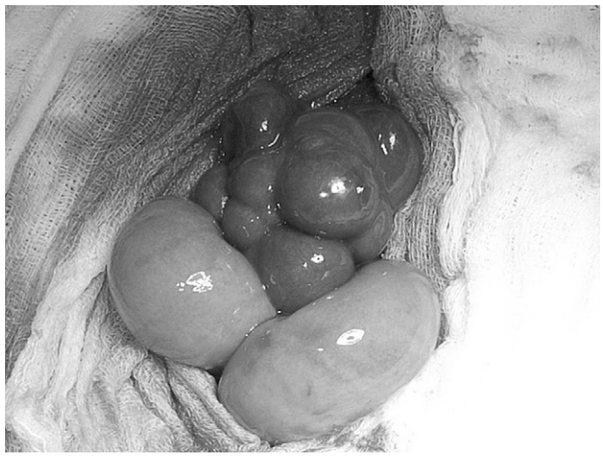

Fig. 124-19 Surgical embryo transfer collection, corpora lutea.

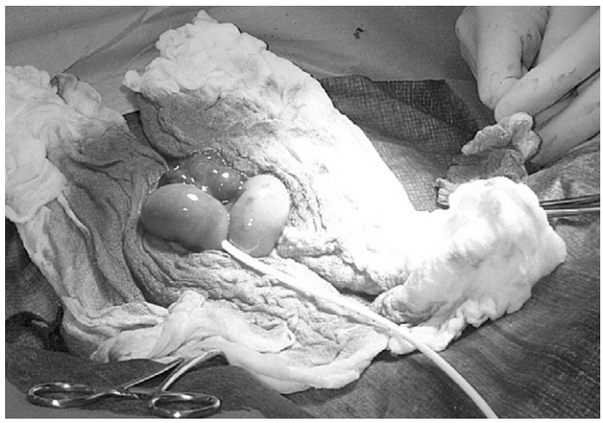

Fig. 124-20 Surgical embryo transfer, uterine flushing.

by inflating the cuff (Fig. 124-20). The uterine horn is flushed from the uterotubal junction toward the base. There is no need to suture the uterus if the suture in the endometrium is not prolapsing through the uterine incision. The linea alba, subcutaneous tissues, and skin are closed routinely as described earlier.

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CHAPTER 125

Reproductive Cycles in Female Cervids

GEOFFREY WILLIAM ASHER

The cervids are a complex assemblage of taxa that include 43 species and 206 subspecies[1] characterized by extreme diversity in morphology, physiology, ecology, and geographic distribution. It is important to recognize that in any consideration of reproductive functions no one species represents a "typical" deer. For example, some cervids exhibit highly seasonal patterns of births in cool temperate climes, and others are completely aseasonal in equatorial regions. Furthermore, many species are strictly monovular and bear single offspring annually, whereas others are normally polyovular and bear multiple offspring.

An increased understanding of reproductive function in cervids over the last few decades stems largely from the growing importance of various deer species in agriculture (e.g., deer farming) and ex situ conservation efforts (e.g., captive breeding of endangered species). In general, the research has been directed toward successful application of assisted reproductive technologies to manipulate the genetic constitution of populations (e.g., selection for improved performance traits; avoidance of inbreeding depression) or to increase rates of propagation of specific genetic lineages. There is also a growing awareness of understanding reproductive patterns to assist in the management of under- or overabundant wild deer populations.

However, it should be emphasized that studies to date have focused on a few key species that do not necessarily reflect the full range of cervid physiology. For example, considerable research investment has been directed at the reproductive and genetic management of farmed deer, such as European red deer (*Cervus elaphus* spp. *scoticus, hippelaphus*), North American wapiti (*Cervus elaphus* spp. *nelsoni, roosevelti, manotobensis*), European fallow deer *(Dama dama dama),* chital deer *(Axis axis),* and rusa deer *(Cervus timorensis).* All tend to be genotypes that exhibit notable gregariousness and are adapted to mixed browsing/grazing. Farmed species, therefore, represent a specific subset of the entire cervid genome biased toward the larger-bodied, monovulatory species with a high degree of behavioral plasticity. Many other cervid species not used for agricultural purposes exhibit different behavioral and reproductive characteristics, particularly smaller-bodied, forest-dwelling browsing cervids of tropical regions.

REPRODUCTIVE STRATEGIES

Seasonal Breeding

The necessity for most cervid species to give birth at an appropriate time of year for optimal survival and growth of offspring has exerted considerable influence on their reproductive physiology. Species of northern temperate origin, which constitute the majority of farmed deer, typically conceive in autumn and calve in summer, whereas species of tropical origin exhibit limited seasonality or are completely aseasonal.[2]

The endogenous mechanisms governing seasonal reproductive patterns in temperate species are robust, being manifest rigorously when animals are transferred between localities despite subtle variations in climate and seasonal feed supply. Furthermore, transference across the equator results in an exact 6-month phase change, even though the relationship between season and feed production differs considerably between continental Northern Hemisphere and insular Southern Hemisphere environments.[3]

It is now well established that entrainment of seasonal reproductive cycles of temperate cervid species is affected by endogenous recognition of photoperiodic changes, with the majority of species initiating mating activity during decreasing day length of late summer and autumn. Variations in the actual mating season between species of up to 8 weeks usually offsets genetically determined species differences in duration of gestation, such that parturition in most species occurs in midsummer.

The translocation of cervid species outside their natural latitudinal ranges is often associated with reproductive maladaptation to the new environment, even after decades of acclimatization. There have been recent attempts to establish temperate species, such as red deer and fallow deer, in equatorial zones (despite the presence of locally adapted cervid species). Anecdotal evidence indicates that removal of photoperiodic signals has had a marked effect on the overall physiology of these deer. For example, fallow deer translocated from New Zealand to southeast Asia exhibited apparent "desynchronization" to the extent that 70% of does became anovulatory at the time of joining during their "normal"

breeding season. This situation was subsequently corrected by strategic administration of melatonin implants for periods of 2 to 3 months.

Even the translocation of temperate species back into different temperate environments can be associated with a degree of reproductive maladaptation. For example, red deer and fallow deer of northern continental origin have frequently been translocated to Southern Hemisphere environments over the last century. Despite this period of acclimatization and the obvious successes in colonizing new habitats, reproductive patterns still strongly adhere to ancestral adaptations. Such reproductive seasonality has obvious beneficial consequences for the species within their traditional environment. However, translocation to more moderate, insular Austral environments has been associated with a degree of misalignment between reproductive seasonality and seasonal changes in feed availability. This has been particularly noticeable for deer farmed on pastoral environments in which peak feed production generally occurs 2 to 3 months before calving/lactation.[3]

Prolificacy and Fecundity

With few exceptions, cervid species generally reproduce annually, irrespective of whether they are seasonal or aseasonal breeders. Therefore, variation between species in reproductive prolificacy largely reflects species differences in fecundity (e.g., ovulation rate, litter size). Some species are strictly monotocous (bear singleton offspring) and others are polytocous (capable of bearing multiple offspring). This divergence in reproductive productivity has been linked to the overall life strategies of the various species, based on the principles of "r" and "k" selection.[4] In general, the monotocous "k-selected" species represent the larger-bodied, long-lived, later-maturing roughage feeders (i.e., grazers) and include most species within the genera *Cervus* (red deer, wapiti), *Dama* (fallow deer), *Axis* (chital deer), and *Rangifer* (reindeer, caribou). These species characteristically form stable populations within climax vegetation zones (e.g., grasslands). Although twinning has been recorded in these species, its incidence is generally below 1% of all births.

The polytocous "r-selected" species generally represent the smaller-bodied (moose are the obvious exception), shorter-lived, early-maturing selective browser/concentrate feeders and include most species within the genera *Odocoileus* (white-tailed deer, black-tailed deer, mule deer), *Alces* (moose), *Capreolus* (roe deer), etc. These species are characterized by their ability to rapidly infiltrate and populate disturbed habitats (e.g., seral vegetation communities) but are generally displaced within climax communities. In many such species, twins and triplets are the norm if environmental conditions (e.g., feed supply) favor investment into high reproductive rates (e.g., white-tailed deer, *Odocoileus virginianus*). These species also tend to be short-lived and are generally more susceptible to diseases than "k-selected" species.

ESTRUS AND OVULATION

The processes of estrus and ovulation are closely aligned, both physiologically and temporally, to ensure fertilization of ova. Recent studies of red deer, wapiti, and fallow deer have demonstrated that the temporal sequence of ovarian and endocrine events associated with estrus/ovulation share considerable similarity to those observed in other ruminant species.

The preovulatory period (up to 48 hours before ovulation) is characterized by a number of precisely orchestrated events: (1) the demise of luteal tissue (luteolysis) from a previous ovulation (often a "silent" ovulation, see later discussion) that causes a rapid decline in blood progesterone concentrations; (2) the emergence of a dominant estrogenic follicle that progressively secretes estradiol into the peripheral circulation; and (3) a subtle but perceptible change in the dynamics of pituitary secretion of luteinizing hormone (LH) in response to follicular estradiol secretion.

The onset of behavioral estrus in all species studied coincides with a marked elevation in plasma estradiol concentrations in the peripheral blood and the onset of the preovulatory LH surge (i.e., a massive, sustained increase in plasma LH concentrations that ultimately leads to follicular rupture/ovulation) (Fig. 125-1). It is highly likely that, as in other ruminants, secretion of estradiol by the emergent follicles attains a plasma threshold that instigates both estrus and the LH surge. Such elevations in peripheral blood have also been observed in white-tailed deer, reindeer, and axis deer. In fallow deer, in addition in increased estradiol secretion, a notable increase in plasma androstenedione concentrations has been observed at the onset of estrus. This weak androgen is also likely to be of follicular origin and may have a role in estrous behavior in this species.

Although behavioral estrus is usually terminated at copulation (i.e., within a hour of onset) in studied cervid species, the preovulatory LH surge persists for ~18 hours, reaching a peak 10- to 20-fold higher than basal values, 3 to 8 hours after the onset of estrus (see Fig. 125-1). The LH surge is a mandatory prerequisite for follicular rupture, which has been timed in both red deer and fallow deer to occur 24 hours after the onset of estrus/LH surge. This timing is in very close agreement with studies on sheep and cattle.

Estrous Cycle

With few exceptions (e.g., roe deer), female cervids are polyestrous, and nonpregnant animals are capable of exhibiting either continuous estrous cycles (e.g., some tropical species) or, more commonly, alternating periods of estrous cyclicity and anestrus (Fig. 125-2). Estrous cycles have been characterized for a number of cervid species based on studies on recurrent estrous behavior and luteal secretion of progesterone in nonpregnant females (Table 125-1).

The estrous cycle consists of a number of discrete luteal events of highly specific duration. Although there is a high degree of similarity in luteal events among various cervid species, there are subtle variations in the time courses that give rise to differences in species–specific estrous cycle lengths (i.e., the interval between two successive estrous episodes) (see Table 125-1).

Luteinization of postovulatory follicles (i.e., luteal phase) is associated with increased secretion of

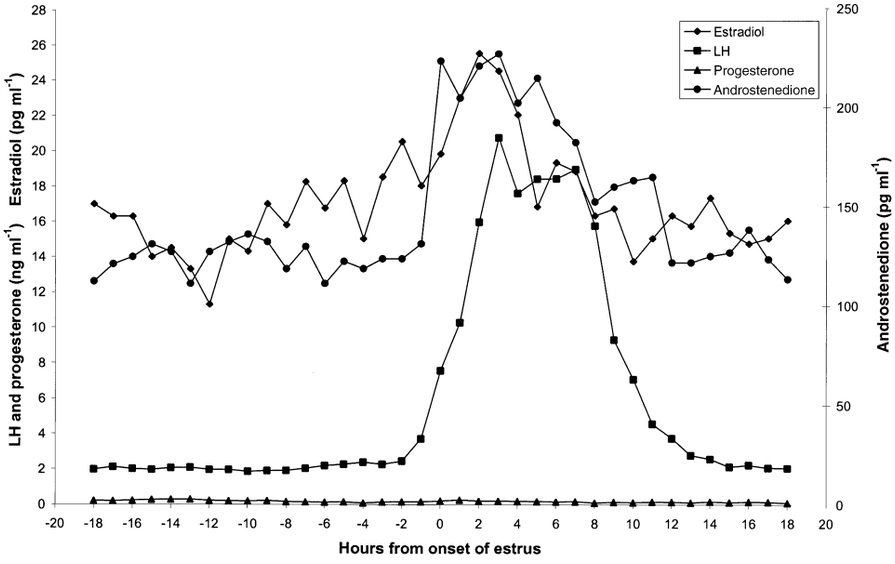

Fig. 125-1 Mean plasma concentrations of luteinizing hormone (■), progesterone (▲), estradiol (◆), and androstenedione (●) around the onset of estrus of a fallow deer doe. (From Asher GW, Barrell GK, Peterson AJ: Hormonal changes around estrus of farmed fallow deer, *Dama dama*. *J Reprod Fertil* 1986;78:487–596.)

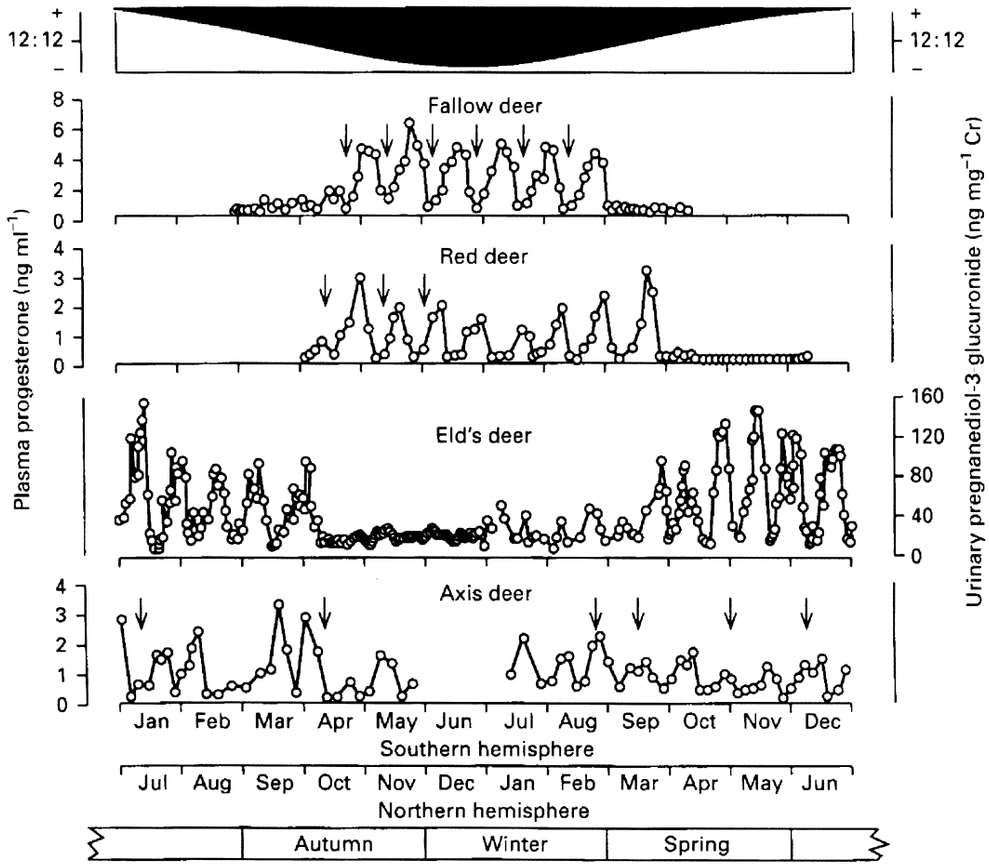

Fig. 125-2 Estrous/luteal cycles in temperate (fallow deer, red deer) and tropical (Eld's deer, chital deer) species in temperate environments, as defined by peripheral plasma progesterone concentrations or urinary pregnanediol metabolite concentrations during the annual cycle of nonpregnant females. The data have been normalized about common hemispheres and placed in relation to relative annual changes in photoperiod. Arrows indicate observed estrous behavior. (From Asher GW, Montfort SL, Wemmer C: Comparative reproductive function in cervids: implications for management of farm and zoo populations. *J Reprod Fertil Suppl* 1999;54:143–156.)

Table **125-1**

Summary of Reproductive Parameters for Various Cervid Species

Species	Common Name	First Estrus of Season*	Estrous Cycle Length (Days)	Potential Cyclicity (Months)	Birth Period*	Gestation Length (Days)	Notes
Cervus elaphus	Red deer	Oct	18–19	5	June	234	Estrous cycle length increases during the season
Cervus elaphus	Wapiti	Sept/Oct	19–20	5	June	247	
Cervus elaphus	Red deer × wapiti	Sept/Oct	19	5–6	June	240	
Elaphurus davidianus	Pere David's deer	Aug	19.5	5–6	May	284	Unusually long gestation length
E. davidianus × *C. elaphus*	PD × red deer	Sept/Oct	19.6	5	June	240–274	Highly variable gestation length
Dama dama dama	European fallow deer	Oct	21–24	5–6	June	234	Estrous cycle length increases during the season
D. d. mesopotamica	Mesopotamian fallow deer	Sept	20–22	—	May	~234	
D. d. dama × *D. d. mesopotamica*	Fallow deer hybrids	Sept/Oct	20–23	5–6	May/June	226–240	
Cervus unicolor	Sambar deer	Jan–March	20	4–5	Oct–Dec	247	Tropical species studied in temperate zones
Cervus timorensis	Rusa deer	Jan–March	18	—	Oct–Dec	249	Tropical species studied in temperate zones
Cervus eldi	Eld's deer	Jan–March	18.5	7.8	Aug–Oct	236	Tropical species studied in temperate zones
Axis axis	Chital deer	All months	18–19	Year round	Year round	235	Tropical species studied in temperate zones
Cervus nippon	Sika deer	Oct/Nov	21	—	June	224	
Odocoileus virginianus	White-tailed deer	Oct/Nov	21–30	6–7	May/June	200	Species occupies a wide latitudinal zone
Odocoileus hemionus	Black-tailed deer	Nov	21–26	4–5	June	201	Estrous cycle length increases during the season
Alces alces	Moose	Oct	24	5–6	May/June	231	North American subspecies
Capreolus capreolus	Roe deer	July/Aug	Monovulatory	—	May/June	259–300	Exhibits delayed implantation
Rangifer tarandus	Reindeer	Sept/Oct	21	>5	May/June	216	Domesticated form of caribou
Muntiacus reevsi	Reeve's muntjac	All months	14–15	Year round	Year round	210	Tropical species studied in temperate zones
Pudu pudu	Pudu	Sept/Nov	~11	—	April/Aug	200	Wide breeding seasons

*Season given as Northern Hemisphere equivalent for species studied in the Southern Hemisphere.
Data in table compiled from multiple reference sources.

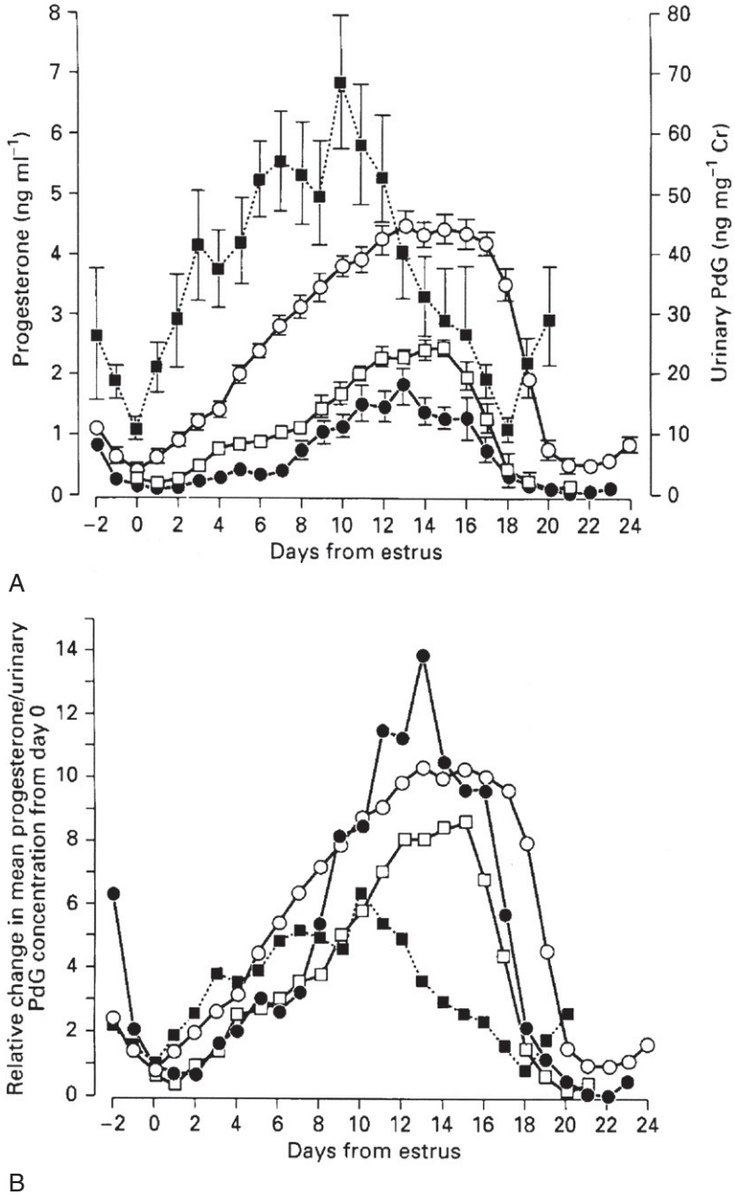

Fig. 125-3 Profiles of mean peripheral plasma concentrations of progesterone *(solid line)* or urinary concentrations of pregnanediol metabolite *(dashed line)* during the cervid estrous cycle for fallow deer (○), red deer (□), axis deer (●), and Eld's deer (■). **A,** Mean (±SEM) values. **B,** The same data presented as relative change in mean concentrations from day 0. (From Asher GW, Montfort SL, Wemmer C: Comparative reproductive function in cervids: implications for management of farm and zoo populations. *J Reprod Fertil Suppl* 1999;54: 143–156.)

progesterone, with maximal output occurring between days 10 and 16 of the estrous cycle (day 0 = estrus). In the absence of a preimplantation embryo, the new corpus luteum then undergoes luteolysis between days 16 and 23 in response to an interplay between luteal secretion of oxytocin and uterine secretion of prostaglandin $F_{2\alpha}$ (as demonstrated for red deer, fallow deer, and reindeer). This causes a rapid drop in peripheral blood progesterone levels, leading ultimately to estrus/ovulation. Although absolute plasma progesterone concentrations during the estrous cycle vary among species (Fig. 125-3, *A*), the relative changes from day 0 are quite similar between species (Fig. 125-3, *B*). Average duration of the normal estrous cycle ranges from 17 days in some tropical species of deer to 18 to 20 days in red deer, 21 to 23 days in fallow deer, and 24 to 27 days in moose and black-tailed deer (see Table 125-1). The mean duration of the estrous cycle tends to increase progressively during the breeding season in red deer, fallow deer, and black-tailed deer. In a number of species occasional "long-cycles" (two to three times normal duration) have been observed, but their significance is unknown.

The transition into the breeding season is characterized by "silent ovulations" (i.e., ovulations not preceded by overt estrus) and short-lived (8–10 days) corpora lutea in most species studied. In fallow deer, multiple successive silent ovulations leading up to the start of the breeding season have been observed. The transient nature of the preliminary corpora lutea may actually serve to promote within-herd synchrony of first overt estrus of the season.

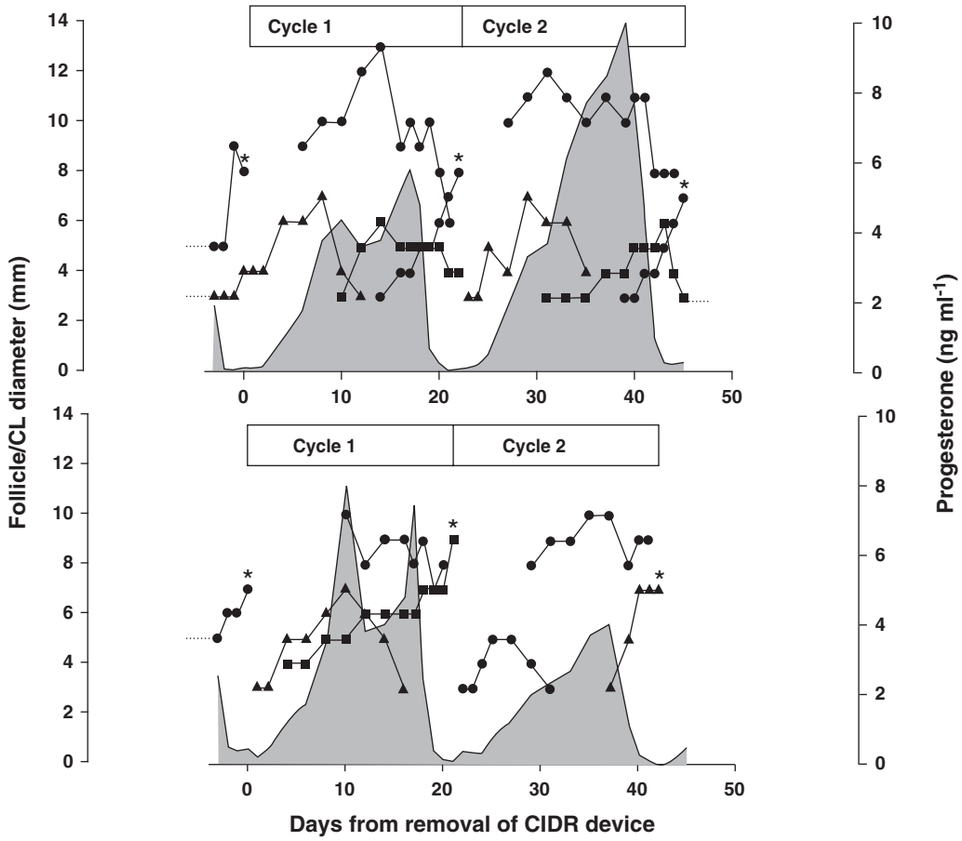

Fig. 125-4 Profiles for fallow deer does of plasma concentrations of progesterone *(shaded profiles)* and diameters of corpora lutea (○, ▲, ■) during two consecutive estrous cycles. Asterisks denote abrupt follicular disappearance indicative of ovulation. (From Asher GW, Montfort SL, Wemmer C: Comparative reproductive function in cervids: implications for management of farm and zoo populations. *J Reprod Fertil Suppl* 1999;54:143–156.)

When considering cervid estrous cycles it is important to maintain the perspective that continuous estrous cyclicity is not a normal phenomenon for most female cervids, particularly those species that exhibit highly seasonal breeding seasons. Although a return to estrus/ovulation following conception failure increases opportunities to establish a later pregnancy, evolutionary pressures for maintaining a highly synchronous calving season at an optimal time of year are very rigorous. The penalties, in terms of neonate death, for conceiving and calving later in the season are often high, particularly in harsh environments. Therefore, many seasonally breeding cervids exhibit exceptionally high rates of conception to their first estrus of the breeding season (~85% in red deer and fallow deer), and subsequently terminate further ovulatory activity for the season.

Ovarian Follicular Dynamics

The patterns of ovarian follicular turnover during the estrous cycle and anestrous period have been described from recent ultrasonographic studies on red deer, wapiti, and fallow deer. Understanding these patterns will assist in future development and fine tuning of assisted reproductive technologies.

Follicle changes during the estrous cycle of deer have shown considerable similarity to those of cattle. Discrete, nonrandom patterns of follicle emergence, growth, and regression are indicative of the existence of (1) strong dominance by a single follicle and (2) discrete dominant follicle waves within the estrous cycle. In the case of red deer, normal estrous cycles were characterized by a variable number (one to three) of dominant follicle waves within each cycle, with only the final dominant follicle present during luteolysis ovulating. In fallow deer and wapiti, estrous cycles were observed to have two to four dominant follicle waves (Fig. 125-4), with the number of waves correlated with cycle length (ranging from 21–23 days). Emergence of new small follicles generally occurred only following the demise of the larger dominant follicles, particularly after ovulation (i.e., during the early luteal phase of the new estrous cycle) when progesterone secretion was minimal.

The anestrous period in nonpregnant red deer is generally characterized by anovulation, the complete absence of luteal tissue and minimal blood progesterone concen-

trations (exceptions to this include persistent corpora lutea remaining from the end of the breeding season in some nonpregnant hinds). However, anestrus, from late spring through to early autumn, is a very dynamic state, as evidenced from studies on pituitary LH secretion. This is reflected strongly in patterns of follicle turnover. Ultrasonographic scanning in early, mid-, and late anestrus has revealed markedly differing patterns of follicle emergence, growth, and regression. In particular, mid-anestrus appears to be a state of more rapid turnover of smaller follicles than during periods of transition in or out of the breeding season. The implications of such observations on control of seasonal breeding patterns remain to be seen.

PREGNANCY AND PARTURITION

Fertilization and Embryonic Development

Few studies have focused on aspects of fertilization and early embryonic development in deer. Recent research on red deer reflects the growing importance of this species in agriculture. These studies have largely sought to characterize the natural (in vivo) developmental sequences (Table 125-2) in order to successfully develop assisted reproductive technologies (e.g., embryo transfer, IVEP) to facilitate genetic management of farmed red deer.

In red deer, oocyte nuclear maturation prior to ovulation appears to be similar to that of other ruminant species. Oocytes are ovulated at the MII stage 20 to 24 hours after the onset of the preovulatory LH surge. A polar body is clearly visible in the perivitelline space. The surrounding *corona radiata* cells appear to be lost within 6 hours of ovulation.

Motile spermatozoa have been seen in the red deer oviduct as early as 8 hours after coitus, but probably actually reach the oviduct within minutes of mating. Fertilization and pronuclei formation are quite rapid. As with cattle, syngamy has been observed within 13 hours of ovulation.

Embryonic development up to days 5 to 6 from estrus (i.e., up to 16 cell/morula stages; see Table 125-2) occurs in the oviduct. Thereafter, the embryo enters the uterus to complete its development. This is later than occurs in sheep but is similar to horses. Subsequent embryo

Table **125-2**

Sequence of Events and Embryonic Development in Red Deer

Time from Onset of Estrus	EVENT/CONCEPTUS STAGE		
	Study 1	*Study 2*	*Study 3*
20–24 h	Ovulation	—	—
29–33 h	Fertilization	—	—
30–38 h	Male and female pronuclei formed	—	—
90 h (4 d)	8-cell embryo	—	—
124 h (5 d)	16-cell embryo	—	—
137 h (6 d)	Compact morula	—	—
180 h (8 d)	Early blastocyst	—	—
190 h (9 d)	Expanded blastocyst	—	—
11 d	—	Spherical blastocyst	—
12 d	—	—	Hatched blastocyst
14 d	—	Rapid expansion, filamentous embryo	Rapid expansion, development of embryonic disc
16 d	—	Trophoblast invades contralateral horn; embryonic disc	Rapid elongation of embryo
18 d	—	Trophoblast 26–30 cm long	Early-late filamentous stage
20 d	—	—	Embryo filamentous and adhered to uterus
21 d	—	Expansion complete; trophoblast fully attached; yolk sac vascularized; two-chambered heart beating	—
23 d	—	Allantois 0.6 ± 0.17 (SE) cm; 19–23 somite pairs	—
25 d	—	Amnion prominent; allantois 3.45 ± 0.51 (SE) cm; brain differentiation	—

Data taken from three separate studies between 0 and 25 days from mating:

1. Berg DK, Thompson JG, Peterson AJ, Asher GW: Morphology and chronology of pre-implantation embryonic development of red deer *(Cervus elaphus).* Proceedings of the Third International Congress on the Biology of Deer, 1996, pp 179–180.

2. Peterson AJ, Ledgard AM, Berg DK: Conceptus development and associated fertility in red deer *(Cervus elaphus)* from days 11 to 25 after mating. *Theriogenology* 1997;47(1):402.

3. Demmers KJ, Jabbour HN, Deakin DW, Flint APF: Production of interferon by red deer *(Cervus elaphus)* conceptuses, and the effects of roIFN-t on the timing of luteolysis and the success of asynchronous embryo transfer. *J Reprod Fertil* 2000;118:387–395.

development (see Table 125-2) follows a similar pattern to other ruminant species.

Interestingly, conception rates in red deer are some of the highest recorded for domesticated ruminants, being in the order of 80% to 85% within 7 days of mating. Furthermore, subsequent embryo survival rates up to 20 days from mating are very high (probably >95%). No distinguishing features of early embryonic development explain the differing fertility among ruminant species.

Maternal Recognition of Pregnancy

Almost all cervids exhibit estrous cycles that may be terminated by successful conception. Repeated cycles reflect cyclic formation and destruction of corpora lutea, whereas pregnancy blocks the luteolytic process and allows persistence of corpora lutea. Luteolysis is the result of interplay between the hormones oxytocin and prostaglandin. In red deer, the end of the luteal phase (days 16–18 of the estrous cycle) is associated with increased uterine oxytocin sensitivity leading to a cascade of pulsatile release of oxytocin and prostaglandin $F_{2\alpha}$. This ultimately precipitates luteal regression and the concomitant decline in progesterone secretion. The pulsatile release of these two hormones is suppressed in the presence of a preimplantation embryo, presumably as a consequence of the release of an embryonic signal. As in other ungulate species, this signal is believed to be a specific form of interferon. This is supported by studies showing that uterine flushings from pregnant red deer hinds contain interferon and the preimplantation embryo secretes the interferon molecule following in vitro culture for 24 hours. In addition, administration of exogenous interferon in the mid-late luteal phase of the estrous cycle extends the luteal lifespan by suppressing uterine oxytocin sensitivity and pulsatile release of prostaglandin $F_{2\alpha}$.[11]

Postconception Ovulation

Postconception ovulation, leading to the development of accessory corpora lutea, is a phenomenon observed in red deer, wapiti, white-tailed deer, and sika, but does not appear to have been seen in fallow deer. Its incidence is particularly high in wapiti (~60–80% of pregnancies). Accessory corpora lutea are noticeably younger than corpora lutea derived from the "estrous" ovulation, and appear to form from ovulations occurring during the later preimplantation embryo development phases. The mechanisms and functions of postconception ovulation and luteal development are unknown for deer. It does seem likely, however, that the additional luteal tissue assists in pregnancy maintenance by increasing circulating peripheral concentrations of progesterone.

Embryonic Implantation

Based on two studies on red deer only, embryonic implantation in cervids occurs about 20 to 21 days after mating. At this stage of development, embryonic expansion is complete and the trophoblast is in close apposition over the entire surface of the endometrium and the head and tail regions of the neural tube remain open with 12 to 15 pairs of somites. The yolk sac has vascularized and a two-chambered beating heart can be observed. The allantois is just visible as a club-like protuberance from the hindgut (see Table 125-2).

Placentation and Fetal Development

Ruminant placentation is typically superficial (the blastocyst elongates, filling both uterine horns, and the trophoblast attaches to the uterine epithelium) and polycotyledonary. In most cervid species studied, placentation is oligocotyledonary, with 6 to 12 placentomes formed, and synepitheliochorial (six tissue layers separate the maternal and fetal circulation). Fetal and placental growth has been most intensively studied in red deer, based on accounts of dissection and real-time ultrasonography.[12,13] The gestational period from days 27 to 55 is arguably the most interesting in terms of fetal development and placentation.

Following embryonic implantation around 20 to 21 days from mating, the trophoblast expands and infiltrates both uterine horns. By day 27 the fetus is about 6 mm long and has a discernible two-chambered heart. The amnion and yolk sac are present, but there is little evidence of placentomes at this stage. By day 34, at about 13 mm in length, the fetus has undergone considerable development, with limb buds and head prominent. The yolk sac has diminished and the allantois fills the entire chorion. Plaques are appearing on the trophoblast, adjacent to caruncles. Erythrocytes can be seen invading the caruncular area. By day 41, at about 28 mm crown-rump length, the fetus shows clear evidence of skeletal primordia. The heart is now four-chambered and the stomach (with ruminant chambers) is emerging. The metanephros and gonads are also evident. Cotyledons have formed on the trophoblast, with cotyledonary villi penetrating developing caruncular crypts. By day 48, at about 38 mm crown-rump length, the fetus exhibits considerable lengthening of the limbs, and has pronounced snout, eyelids, and hooves. The gonads are large but undifferentiated, and the adrenal glands are clearly evident. Placentomes have formed in most caruncles and some additional placentomes have formed. By day 55, at about 55 mm in length, the fetus is very "deer-like" in appearance. The limbs and skull have lengthened, the sex organs have differentiated, and the kidneys are evident. There are also prominent pedicle swellings becoming apparent on the skull (i.e., pedicle/antler primordia). Placentation is well developed by this stage, with the full complement of placentomes present (although the actual number varies with each conceptus).

It seems likely that the aforementioned pattern of red deer fetal and placental development within the 80 to 90 days of pregnancy is similar across cervid species, irrespective of adult livemass. This is certainly true for fallow deer, which are less than 50% of the adult livemass of red deer, but exhibit virtually identical growth of the fetus in the first 55 days of pregnancy.

Fetal and placental development in the latter stages of pregnancy have, again, been well documented for red deer, and appear to follow patterns that are fairly typical

for ruminants. These have been described as Gompertz equations of growth for gestational age.[14] As cervids in general share a degree of commonality in gestation length (i.e., 200–250 days; see Table 125-1), it is likely that fetal and placental growth follow similar patterns across species, albeit different trajectories according to species-specific birth weight ranges. As is typical of all ruminant pregnancies, conceptus growth trajectory is most rapid during the last third of pregnancy.

Although twinning is uncommon for red deer, there are numerous documented cases in both wild and farmed populations (many of those among farmed red deer have been artificially induced with exogenous gonadotropins during artificial breeding programs). It has recently become apparent that female red deer calves born from disex pairs exhibit a high degree of freemartinism. This indicates a degree of shared fetal placentation in red deer. However, naturally twinning species (e.g., white-tailed deer) do not exhibit freemartinism, suggesting a trend toward complete isolation of fetal placentation.

Although trends in fetal growth trajectory have been generalized for red deer, it is important to recognize the possible influences of extrinsic factors on rates of fetal development, particularly during the last third of pregnancy. For example, recent studies have shown that the level of nutrition to hinds during late pregnancy significantly influences fetal/conceptus growth rate (see later). Also, there may be pronounced intrinsic (e.g., genetic) influences, particularly in the case of hybridization between taxa (e.g., red deer × wapiti hybrids).

Role of the Corpus Luteum during Pregnancy

There is variation among ruminant species as to the role of the corpus luteum in maintaining the viability of pregnancy. Some species (e.g., goats) rely entirely on luteal production of progesterone throughout pregnancy, and others (e.g., sheep) instigate placental synthesis that supersedes ovarian production within the first trimester of pregnancy. Little is known about the influence of luteal and placental progesterone on pregnancy in cervids. Numerous studies across a number of cervid species have demonstrated physiologically significant quantities of progesterone in the peripheral circulatory system during the entire term of pregnancy. However, the relative contributory roles of luteal and placental tissues in its synthesis and secretion have yet to be fully determined.

In white-tailed deer, ovariectomy during various stages of pregnancy invariably resulted in fetal death and abortion within 48 to 96 hours. However, in red deer, wapiti, and reindeer, ovariectomy or luteectomy (removal of the corpus luteum) resulted in abortion in most, but not all, pregnant females. This indicates the possibility of extraovarian sources of progesterone supporting pregnancy.

Treatment of pregnant red deer and reindeer hinds with prostaglandins again caused fetal loss in most, but not all, cases. However, failure to abort may have been due to incomplete luteal regression and subsequent luteal regeneration. This indicates a degree of luteal refractoriness to prostaglandins during pregnancy, rather than indicating an extraovarian source of progesterone synthesis.

Gestation Length

Species-specific gestation lengths tend to be positively correlated with adult body mass in mammals generally. Although known gestation lengths for various cervid species (see Table 125-1) are also correlated to body size, cervid gestation lengths are relatively long (200–250 days) compared to those for other ruminants of similar size (e.g., sheep and goat species; ~150 days). The imperatives of highly synchronized annual reproduction necessitate prewinter rutting/conceptions and summer calving, the consequence of which is a relatively long gestation. The fact that one species, the roe deer, employs delayed implantation in order to "extend" gestation length highlights the importance of appropriate seasonality of conceptions and birth.

Although gestation length is clearly under a high degree of genetic control, variation in actual lengths are evident within each species. The highly variable gestation lengths recorded for hybrids between red deer and Pere David's (PD) deer indicates strongly that relatively few genes control species-specific gestation length. The two parental species have widely differing pregnancy durations (234 versus 284 days), and the backcross hybrids (e.g., 75% red deer, 25% PD deer) exhibit very wide variation (240–274 days) typically seen in genetic traits in which there is random segregation of alleles at relatively few gene loci.

However, there is little doubt that various extrinsic factors contribute to some variation in gestation length. For example, the average length for European red deer is 234 days. However, the observed range is 224 to 250 days. Recent studies on pregnant red deer hinds have shown that the level of nutrition over the last third of pregnancy can influence gestation length in this species. Hinds under moderate nutritional stress exhibited retardation of conceptus growth but appeared to compensate for poor fetal development by delaying parturition by up to 7 to 15 days. In this way they appeared able to maintain viable calf birth weights. The mechanisms by which this occurs remain unknown but may involve an effect of fetal growth rate on maturation of the fetal adrenal glands.

Parturition and Lactogenesis

Little is known about the physiologic mechanisms controlling parturition in cervids. Studies on a number of species (red deer, fallow deer, white-tailed deer) have shown a precipitous decline in blood progesterone concentrations in pregnant females around the day of parturition, reflecting demise of the corpus luteum or placental progesterone source. An elevation in blood estrogen levels has also been observed around birth in white-tailed deer.

There is some evidence that parturition is mediated, at least partly, by the fetus. Red deer hinds carrying wapiti × red deer or PD deer × red deer fetuses tend to exhibit longer gestation lengths than hinds carrying red deer fetuses. Pregnancy durations tend to be intermediate between the gestation lengths to parental taxa, indicating fetal expression of birth processes. In other examples of more extreme hybridization of red deer involving

species of different karyotype (e.g., sambar deer, rusa deer), hinds carrying hybrid fetuses generally exhibit excessively long gestations (i.e., often exceeding the mean length of both parental species by 10–30 days), failure of lactogenesis, dystocia, and high levels of neonate nonviability. This suggests a complex role of the fetus in parturition that is severely perturbed by hybridization.

Lactogenesis occurs in red deer and fallow deer very late in pregnancy, with females showing virtually no palpable or visible udder development before 30 to 40 days before parturition. First calving (i.e., pubertal) red deer hinds may show no signs of udder development until just 3 to 4 days before calving. This renders udder checking an unreliable method of pregnancy diagnosis.

There is growing evidence that lactogenesis in red deer and fallow deer is strongly influenced by prevailing photoperiod (i.e., normally increasing day length) during the last few months of pregnancy. Fallow does subjected to complete reversal of photoperiod during early pregnancy, due to translocation between hemispheres, exhibited a very high level (>95%) of failure of lactogenesis at parturition. Furthermore, treatment of pregnant red deer hinds with melatonin implants starting 80 days before birth (designed to induce an early return to estrus following calving) resulted in complete failure of all hinds to undergo lactogenesis. Interestingly, however, hinds similarly treated from 40 days before birth exhibited full lactation following calving. These observations indicate photoperiod responsiveness of mammary tissues, and that the critical window of lactogenesis lies 40 to 80 days before parturition.

LACTATION

Udder Development and Milk Composition

Irrespective of litter size, the typical cervid udder has four quarters, each with a single teat-canal. In the nonlactating state, the mammary tissue is well regressed into the inguinal region and the four teats are barely visible. However, in the fully lactating state (especially immediately prior to and after parturition), the udder is fully developed to its maximal size and the teats are extended. The udder often extends backward slightly, being very obvious below the vulval region. In red deer, wapiti, and fallow deer, which normally rear singleton offspring, only one or two quarters tend to dominate milk production during the entire lactation.

Little is known about the dynamics of milk production, storage, and let-down in cervids. However, there have been a number of studies on milk composition. Because most deer hide their calves for several weeks after birth, nursing is infrequent and the milk is rich compared with that of large grazers such as cattle (Table 125-3). The colostrum is particularly concentrated and rich in immunoglobulins, which protect the offspring while its immune system is maturing.

Milk production increases to a peak of about 2.5 L per day in red deer and 4.0 L per day in wapiti at about 40 days from birth, declining gradually as long as there is a suckling stimulus.[15] In the wild, this may occur throughout the calf's first winter, although volumes ingested are probably small. Thus, lactation in red deer can last 7 to 9 months in the absence of enforced weaning (as often occurs on deer farms).

Control of Lactational Yields

In red deer, well-fed lactating hinds can produce milk yields of 1400 to 2000 g/day early in lactation. However, nutrition to the hind exerts considerable influence over actual yields, with hinds on restricted food intake producing as little as 50% of the peak yield of hinds on unrestricted intakes.[16] Similarly, range conditions affect feed intake and have considerable bearing on milk production. In one study of red deer in Scotland, hinds on fertilized pasture exhibited milk yields 50% higher than hinds on poor quality hill pastures.[17] This nutritional effect flows through to calf growth rates, and is very well known to red deer and wapiti farmers in New Zealand and Australia. Summer drought conditions in Southern Hemisphere regions often have a negative impact on lactational yields and, ultimately, calf growth.

Other determinants of lactational yields in some cervid species may include genetics of the fetus. It has been a well-established practice in red deer farming in New Zealand to mate red deer hinds with wapiti bulls to produce fast-growing hybrid calves from (relatively) small hinds. The lactational demands on red deer hinds to raise hybrid offspring, which have growth rates twice that of red deer calves, are quite daunting. However, they are

Table **125-3**

Composition of Ruminant Milks

	Solids %	Fat %	Protein %	Lactose %	Ash %	MJ/L %
Cattle	12.6	3.7	3.4	4.8	0.7	3
Sheep	8.4	7.4	5.5	4.8	0.7	5
Bison	13.9	3.5	4.5	5.1	0.8	3.4
Wapiti/red deer	22.3	8.0	8.0	5.0	1.3	5.3
Moose	22.9	10.0	8.4	3.0	1.5	6
White-tailed/mule deer	25.3	10.9	7.6	5.4	1.4	6.7

From Haigh JC, Hudson RJ: *Farming wapiti and red deer.* St. Louis: Mosby, 1993.

capable of doing so if nutrition is not limiting. This suggests that either the hinds are capable of greater milk yields when bearing and raising hybrid offspring or the hybrid calves are capable of greater efficiency of converting milk solids into body growth. In all likelihood, both factors apply, and there is the intriguing possibility that the genotype of the fetus may influence lactogenesis and subsequent lactational yields.

Influence of Lactation on Ovulation

Given the unusually long gestation length of cervids and the imperatives of annual reproduction cycles, the interval between parturition and reestablishment of pregnancy is relatively short (3 to 4 months in most species). As lactation normally persists for up to 7 to 9 months, there is an overlap between calf rearing and reinstigation of ovulatory cycles. The influences of lactation on the incidence and timing of ovulation have received much speculation but are not well studied for cervids. Studies on red deer in Scotland showed that hinds on poor quality pasture had lower milk yields and higher plasma prolactin concentrations than hinds on better quality pasture. The higher prolactin levels were associated with a greater suckling frequency and a later return to estrus compared with the hinds on better pastures.[17] However, this study did not take into account differences in hind body condition (i.e., weight, fatness) that might also influence the onset of ovulatory activity.

Farmed red deer and wapiti are often weaned prior to the autumn rut to facilitate feeding and mating management. This effectively curtails lactation early. Several studies in New Zealand and Canada have compared the effects of pre-rut and post-rut calf weaning on ovulatory activity. In both cases, early cessation of lactation has been associated with advanced ovulatory activity of approximately 12 days in red deer and 5 days in wapiti.[18,19] Although it is difficult to argue that cessation of lactation per se was a causal factor in the breeding advancement, as early weaned hinds generally exhibited dynamic improvements in body condition, there is little doubt that lactation does influence reproduction by some means.

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CHAPTER 126

Reproductive Anatomy and Physiology of Male Wapiti and Red Deer

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REPRODUCTIVE ANATOMY

The reproductive organs of the male members of the genus *Cervus* consist of the testicles, epididymides, ductuli deferentia, accessory glands, and the penis and the prepuce[1–3] (Fig. 126-1). The testicles are located in a dependent scrotum between the hind legs.

The penis is a fairly simple rod-shaped structure that undergoes very little increase in circumference during erection, but increases in length by about 40%.[1] There is no sigmoid flexure (see Fig. 126-1). The urethra, which lies on the ventral surface of the penis through its length, curves dorsally at its extremity, and permits the ejection of urine and a few sperm cells in an upward direction, which is important during "thrash-urination" activity indulged in by rutting males as they spray upward almost at right angles onto their ventral abdomens, neck, and throat[1] (Fig. 126-2).

The prepuce has well developed muscles that are responsible for the remarkable rapid forward and backward movement, often called palpitation, of this region seen during the rut[1] (Fig. 126-3). This behavior, and the degree of development of these muscles, has not been reported from any other genus.

Scrotal circumference increases markedly and peaks at about the time of the onset of the rutting season. A threefold increase in size between early summer and autumn has been noted in red deer stags, and the scrotal circumference of male elk increases by about 50% during this period[4] (Fig. 126-4). After this it declines fairly steadily until it reaches its nadir in spring.[4] The percentage of normal sperm in ejaculates, the scrotal circumference, and the concentrations of serum testosterone undergo annual changes in synchrony.[4]

Cellular changes in the testis and the production of spermatozoa occur seasonally. As testosterone levels rise after the summer solstice, activity within the testicular parenchyma increases. The diameter of seminiferous tubules increases and the development cycle of seminiferous epithelium occurs. As these events take place, so the testicular size changes.[2,4]

At the onset of the rut, the interstitial cells of the testis are of maximum size and number, as is the activity of the seminiferous tubules. When reproductive activity has ceased, in the spring at about the time of antler casting, the interstitial cells are small and inactive, no mature spermatozoa are visible in the lumen, and the cells of the seminiferous epithelium show almost no differentiation beyond the very earliest forms of sperm precursor.[1,2] These changes are reflected in the measurements of scrotal circumference (see Fig. 126-4). Thus, the stag undergoes testicular and hormonal changes similar to puberty on an annual basis.

The epididymis also undergoes seasonal changes. In autumn it is lined by a tall pseudostratified columnar epithelium. By spring the height of this epithelium declines sharply and the nuclei of the epithelial cells are condensed. In autumn the cauda epididymidis is densely packed with sperm. In early summer no sperm are visible in the lumen.[1,2]

Accessory Glands

The accessory glands include paired ampullae, paired vesicular glands, both a body and disseminate part of the prostate, and small bulbourethral glands[3] (Fig. 126-5).

At their distal ends the ductuli deferentia enlarge to form the ampullae. These organs lie in close apposition to one another throughout most of their length in a common bundle of connective tissue. They are 4 to 5 cm long and about 1 cm in diameter at their widest in wapiti. Each ampulla opens separately into the duct of the adjacent seminal vesicle, forming short ejaculatory ducts.[3] Their epithelium exhibits changes that are correlated with the sexual cycle. Histologically the ampullae have the appearance of a branched tubular gland with sac-like dilatations, the crypts of which contained secretion. In May the epithelial height has not changed, but the nuclei have condensed. At this time some crypts contain densely packed degenerative sperm.[1]

The paired seminal vesicles are attached to the anterior end of the pelvic urethra, lateral to the ampullae, and are joined medially by a genital fold. They are 6 to 7 cm long and have a slightly lobulated appearance.[1] In autumn they contain a thick yellow secretion. Each gland opens separately into one of the paired ejaculatory ducts. The glands show increased epithelial height from July into the rutting period in October. They regress by November and contain little or no secretion in spring.[1,2]

The prostate gland consists of two parts. It has a discrete, bilobed body lying anterior to and continuous with

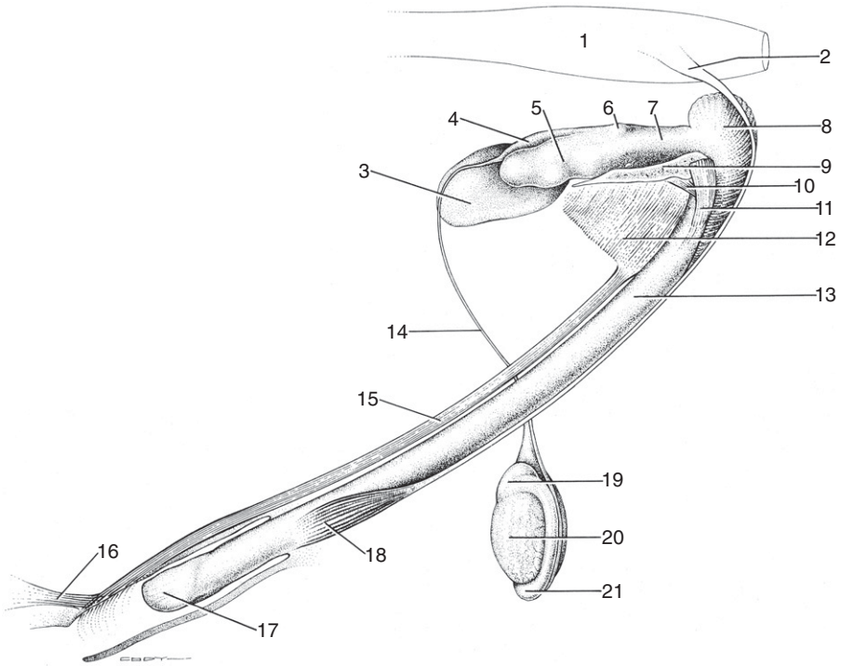
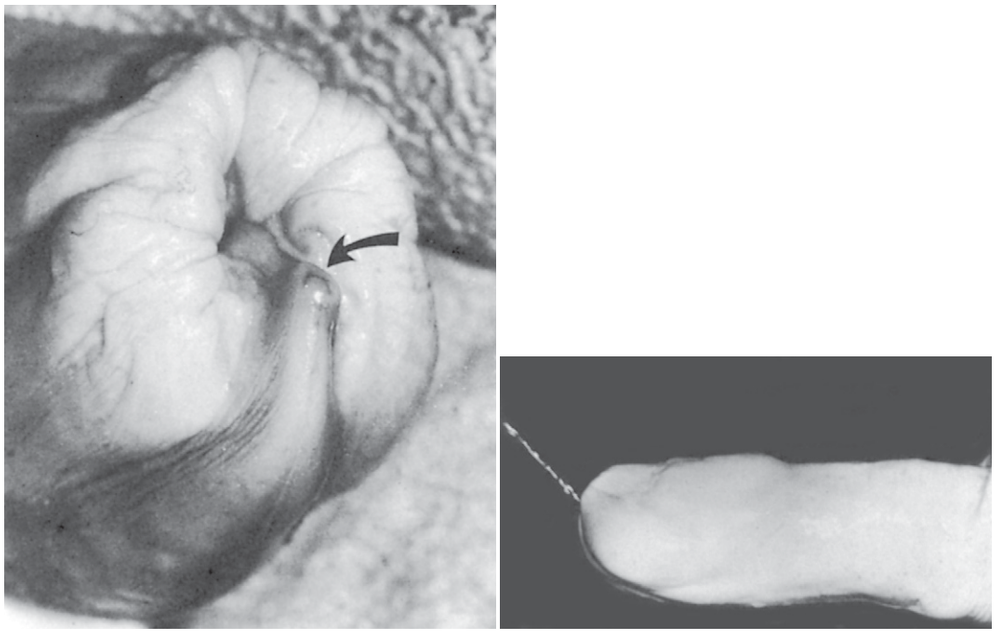


Fig. 126-1 The reproductive tract of the male wapiti. *1*, Rectum; *2*, retractor penis muscle; *3*, urinary bladder; *4*, ampulla; *5*, vesicular gland; *6*, prostate body; *7*, pelvic urethra, also disseminate part of prostate and bulbourethral glands; *8*, bulbospongiosus muscle; *9*, pelvis (cut ischium); *10*, suspensory ligament of penis; *11*, ischiocavernosus muscle; *12*, symphysial tendon; *13*, penis; *14*, vas deferens; *15*, caudal preputial muscle; *16*, cranial preputial muscle; *17* glans penis; *18*, expansion of retractor penis muscle; *19*, caput epididymidis; *20*, testis; *21*, cauda epididymidis. (From Haigh JC, Hudson RJ: *Farming wapiti and red deer.* St. Louis: Mosby, 1993.)



A **B**

Fig. 126-2 Cranial (**A**) and lateral (**B**) views of tip of penis showing upward pointing direction of distal urethra and direction of travel of fluid from urethra when injected under pressure into a more proximal part. (From Haigh JC, Hudson RJ: *Farming wapiti and red deer.* St. Louis: Mosby, 1993.)

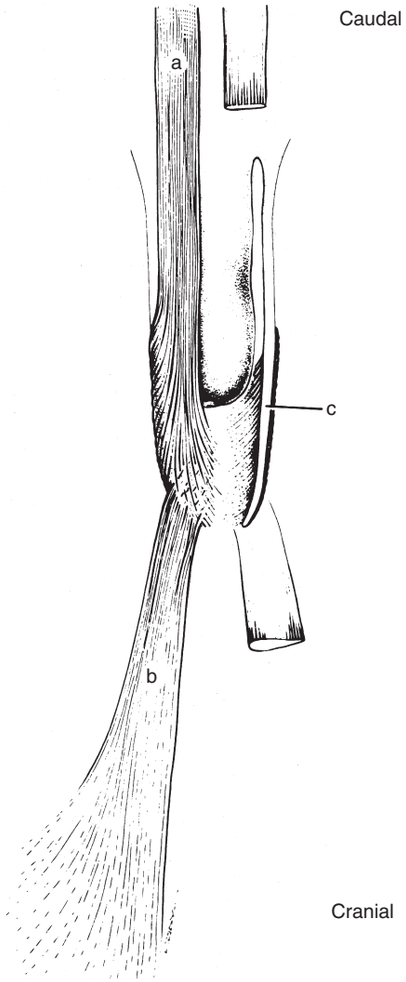


Fig. 126-3 Dorsal view of preputial muscles of wapiti (cut away on one side to show penis and preputial reflection). The cranial muscle (*a*) travels back from a wide insertion and encircles the preputial orifice (*b*). The caudal muscle (*c*) descends from the symphysial tendon and similarly encircles the prepuce. (From Haigh JC, Hudson RJ: *Farming wapiti and red deer.* St. Louis: Mosby, 1993.)

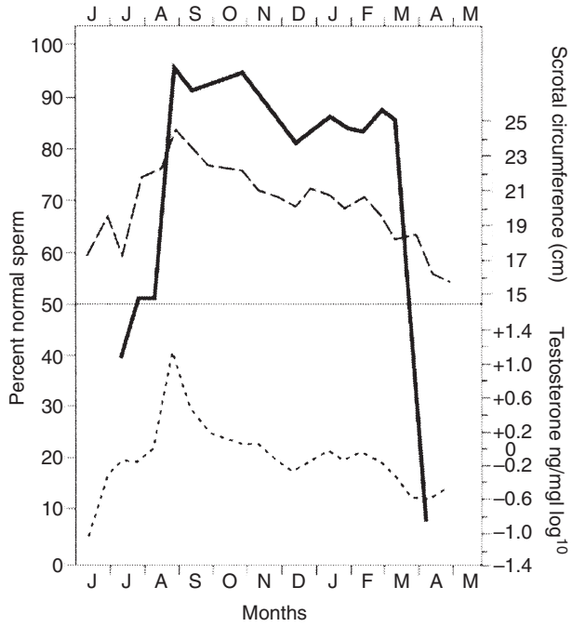


Fig. 126-4 The relationship between month of the year (Northern Hemisphere), percent normal sperm in ejaculates (*solid line*), scrotal circumference (*upper dashed line*), and serum testosterone (*lower dotted line on log scale*) in wapiti. (Redrawn from Haigh JC, Cates WF, Glover GJ, et al: Relationships between seasonal changes in serum testosterone concentrations, scrotal circumference and sperm morphology of male wapiti (*Cervus elaphus*). *J Repro Fertil* 1984;70:413–418.)

the disseminate prostate. This body is visible on the dorsal part of the pelvic urethra as two small discrete hemispherical masses just caudal to the ligamentous band that connects the vesicular glands. It opens ventrally into the pelvic urethra. The epithelium of the prostate body is of a tall, columnar type during the rut and shortens markedly, appearing cuboidal by November.[1,2] The disseminate part of the prostate can be identified throughout most of the pelvic urethra, underlying the urethral muscle. It has numerous ducts opening into the urethra.[1] There appears to be little seasonal change in epithelial height.[1,3]

The bulbourethral glands are extremely small, the combined weight being 0.6g in wapiti. They are found at the junction of the urethral and bulbourethral muscles under a layer of connective tissue on either side of midline. In one study comparing epithelial heights before and after the rut, no differences were found.[3]

Spermatozoa

The spermatozoa of the elk and red deer have the same general appearance as those of other ruminants.[4] Collection of semen on a year-round basis shows that it is possible to obtain ejaculates with a high percentage of abnormal forms in July and August (Northern Hemisphere), but that by the beginning of September ejaculates of mature elk bulls contain a high proportion of normal sperm[4] (see Fig. 126-4). For evaluation of reproductive soundness, and for artificial insemination, semen should be collected from just before the autumnal equinox until a short time after the winter solstice, with highest sperm concentrations expected around and soon after the time of the equinox. From about the time of the spring equinox and for a few weeks afterward wapiti males may be azoospermic. A wide range of spermatozoal abnormalities have been found in both wapiti and red deer that are similar to those seen in domestic bulls, and the same classifications can be used for them.

REPRODUCTIVE CYCLES

All the physiologic events of the reproductive cycle are tied to gestation length, which in wapiti and red deer is timed for delivery of a calf in late spring/early summer.

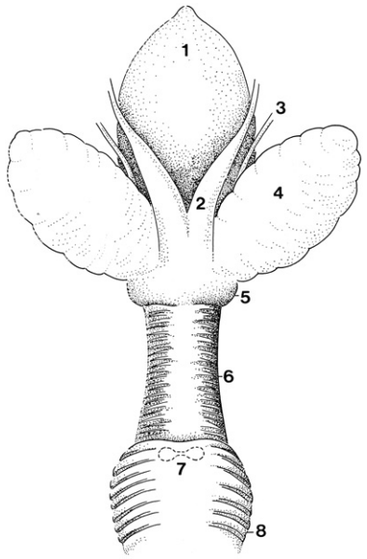


Fig. 126-5 Pelvic organs and accessory glands of the wapiti. *1*, urinary bladder; *2*, ampulla; *3*, ureter; *4*, vesicular gland; *6*, urethral muscle surrounding disseminate prostate and bulbourethral gland distally; *7*, bulbourethral gland; 8, bulbospongiosus muscle. (From Haigh JC, Hudson RJ: *Farming wapiti and red deer.* St. Louis: Mosby, 1993.)

This is late enough to avoid inclement weather and to ensure plenty of high-quality forage, but early enough for the calves to attain sufficient weight to survive the subsequent winter. In the natural ecosystems this occurs at the end of May or early June in the Northern Hemisphere. There are, however, minor modulations in season that are governed by other factors that account for the minor year-to-year variation in rutting and calving dates.[5] Weather or forage quality and availability leading up to the rut are likely factors. Even when members of these species are kept outside the latitude zones in which they evolved they retain the same seasonal breeding pattern.

When these temperate zone deer are taken across the equator they shift their cycles of antler development, breeding, hair coat, appetite, and energy expenditures.[6] This change does not occur immediately, or in all animals at the same time, but usually is complete within 2 years of translocation. The change can be accelerated by the appropriate use of endogenous melatonin.

Puberty

Puberty in the male has been defined as the period when secondary sexual characteristics and accessory organs develop under the influence of the testis.[7] It is a continuous process that begins when the stag has reached a critical weight and is reflected in the initiation of growth of the antler pedicle.. Its onset at this stage is more related to body weight than photoperiod and there are records of undernourished free-ranging red deer not reaching puberty until 3 years of age.[7,8]

Suttie and associates[9] have described the events that occur in the testis of young red deer stags. In the autumn of its first year of life, when the male calf is about 3 months old, testicular diameter begins to increase. The following spring the increase is arrested, but size does not decline. About midsummer at the start of the second year, testicular size again starts to increase as the calf approaches puberty.[9]

From the time that the calf is 3 months of age, pulses of luteinizing hormone (LH) increase to about three per 24-hour period and lead to discrete pulses in testosterone. From the time of the summer solstice the number of pulses of both hormones increases to about eight per day. There is then a decline in spring, and after the spring equinox, by the time that the animals are 11 months old, pulsatile secretion of LH is almost undetectable. After the summer solstice when males were 13 months old, frequent low amplitude pulses of LH are accompanied by frequent discrete pulses of testosterone. In late summer LH frequency is maximal and is accompanied by frequent large-amplitude pulses of testosterone that persists for 2 months.[9]

Although 15-month-old stags may be fertile, they are usually not able to breed successfully in the presence of older males.[7,8] However, in experimental situations they can and will breed hinds in estrus.[10] Indeed, many deer farmers use 1-year-old stags as sires, but with only small numbers of hinds (e.g., 10–15).

Subsequent Cycles

The annual change from an inactive and infertile state to full reproductive readiness has been likened to a seasonal puberty.[2,7,11] The exact details of physiologic activity after puberty have been described in red deer. Elk follow the same pattern, but events occur some 2 weeks earlier as the gestational length is that much longer.

It is also important to note that juvenile animals develop their sexual activity more slowly than do adults, at least until they reach 3 years of age.[2,11,12] When a wapiti reaches the "teen" age (2–5 years) his semen production rises from year to year; more semen can be stored in the epididymis and a progressively greater volume of fertile semen can be ejaculated. These increases continue until the testicle growth is completed and the animal is in its prime.[13] With these facts in mind the sequence of events in an individual mature wapiti bull can be summarized as follows.

At the spring equinox pituitary activity and testosterone concentrations are minimal. At about the summer solstice, the weight of the pituitary gland begins to increase, and with it the output of gonadotropin-releasing hormone (GnRH). Approximately 2 months after the summer solstice pituitary weight is at its maximum, as are LH concentrations. Testosterone concentrations begin to rise in response to the LH activity. Around the autumnal equinox while LH levels are not as high, the responsiveness of the target cells in the testis to LH is much more marked, and serum testosterone levels are at a peak at this time when testicular testosterone concentrations may be 1000 times higher than those measured in spring and early summer. Trials with synthetic

GnRH have shown that the pituitary gland will respond to injections of this hormone at any time of year, but that these responses are greatest just before and at the time of antler velvet cleaning. The consequent testosterone increases occur most markedly at the time of the rut, to a limited extent while the animal is in hard antler, and hardly at all between casting and antler cleaning.[14] Within a month of the autumnal equinox testosterone levels have declined to near baseline levels, where they remain for most of the rest of the winter. Seasonal reproductive changes are more striking in the male than in the female, not least because the most visible secondary sexual characteristic is the presence of antlers, which are used to establish dominance and breeding privileges during the rut. Preceding the rut, testosterone concentrations, scrotal circumference, and percentage of normal sperm increase sharply, reaching a peak just before the rut itself (Fig. 126-4). Other secondary sexual characteristics also change throughout the year. In preparation for rutting battles, the neck swells owing to massive development of the muscles, giving the rutting stag a very distinctive appearance.[11,15]

Although the rapid change in serum testosterone concentrations in later summer is clearly seen, there is a much more subtle and transient elevation after the winter solstice in both wapiti and red deer.[4,16] These elevations correspond to the two annual increases in size of the testicular interstitial cells of red deer.[17] Biannual increases in testosterone have also been seen in several other deer species.

It is commonly observed on deer farms that wapiti and red deer stags that are well nourished will exhibit two rutting seasons a year. The principal one occurs in autumn, and the second one in which some fighting and bugling is heard for a brief period of about 10 to 14 days occurs about 1 month to 6 weeks after the winter solstice. It has been found that the quality of the nutrition influenced the levels of testosterone, and that well-nourished red deer stags can rut twice a year.[16] This is not commonly observed overtly on deer farms, and generally does not cause management problems.

The duration of daylight is the same at this time as it is after the summer solstice, and it may be that the light signal during this period is stimulatory until its increasing duration is recognized and no longer acts as a stimulant. When animals are transferred across the equator the stimuli are reversed.

It has been shown that in the red deer there are seasonal fluctuations of prolactin receptors in the testis and epididymis. The role of prolactin in the testis has not been fully determined, but it may have both stimulatory and inhibitory functions. In the epididymis a receptor was detected only during the breeding and early nonbreeding season.[18]

It is likely that other reproductive hormones also exhibit dual annual cycles. Bubenik and associates[19] reported dual elevations of estradiol-17β in white-tailed deer that occurred in April/May and October/November.

BEHAVIORAL CHANGES

Reproductive behavior is discussed in Chapter 127.

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CHAPTER 127

Reproductive Behavior of Red Deer and Wapiti

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As with other ruminants, female cervids exhibit estrus rather than continual sexual receptivity. In most species, estrus is restricted to the "rutting" season, a period of hypersexual activity during which males contest access to females for mating.[1] Thus, female sexual activity is closely synchronized with that of males, especially in temperate species.*

STAG BEHAVIOR

During the period in which antlers are in velvet stags do not normally engage in any sexual behavior. If stags or bucks are competing while their antlers are in velvet they will normally do so by rising on their hind legs and engaging in boxing activity. However, the presence of hinds in estrus outside the normal breeding season will induce a full range of mounting and mating activity. Normally hinds would not be in estrus at this time, but this type of behavior has been seen when hinds are brought into estrus out of season by artificial hormonal means, or when animals have been transferred across the equator and have not yet adjusted to the new photoperiod. Resumption of rutting behavior occurs late in winter if a female loses a conceptus and begins to cycle again, or begins estrous cyclicity particularly late.[2]

The behavior of stags changes quite abruptly at about the time that antler velvet is shed and antlers become fully calcified. Wapiti gestation is about 2 weeks longer than that of red deer and fallow deer, and so the changes occur that much earlier (for more on the relationship of antlers to reproduction see Chapter 133).[3] They take place, in mature males, about 3 to 4 weeks before the onset of the rut (mating season) itself. Younger males start rutting behavior a little later than mature adults. The behaviors at this time include antler rubbing or thrashing, wallowing, preputial palpitation with or without urine spraying, bugling or roaring, dominance displays, and sparring in wapiti and red deer.[4] At this time, stags become extremely aggressive toward one another and often toward humans in a farming environment. Antler rubbing and thrashing serve to both mark territory and remove the dried velvet tissue on the surface of the hard antler. The thrashing may be accompanied by rubbing of the neck on vegetation, fence posts, or other wooden structures and is probably a form of scent marking. At this time, in male-only mobs, the fighting mode changes from boxing to a complex of displays that may culminate in clashes that involve the use of the head. In single sire mating mobs fighting is not seen, but fence pacing with parallel walking displays projected at males across fence lines, or at people, are common. Males will also face directly up to people at fence lines and carry out head threats that involve a rapid dip. If stags (also called bulls in wapiti) are placed in adjacent paddocks, fighting through the wire will occur.

In *Cervus* males the underside of the belly, the forelegs, and the neck are often darkly stained during the rut and for a period thereafter. This is due to the frequent spraying of fluid from the penis, usually accompanied by preputial palpitation, that is known in elk as "thrash urination," and which is almost certainly used as a scent marker.[4,5] The urethra at the tip of the elk's penis points upward, which allows for a spray that is almost at right angles to its long axis.[6] This ensures that the entire belly and neck can efficiently be scented. The smell of the fluid, and the smell of the stag himself, is particularly strong at this time of year.[4,6] When this fluid has been collected it has not been shown to contain sperm.[7] Thrashing is sometimes accompanied by wallowing. If there is a convenient wet or damp spot, a stag will often choose it for a wallow. He will scrape with his feet in the ground, sometimes will use his antlers, and often will urinate as well. He will then usually roll in the wallow and cover both his belly and neck with the mixture.[4,6]

On deer farms stags have usually been "placed" or "joined" with hinds just before velvet cleaning, or very soon after it occurs in order to take advantage of the well-recognized "stag effect." It has been shown that the stag's rutting behavior advances the onset of estrus if introduced to the female about 3 weeks before onset of estrous cyclicity in the absence of stags.[6,8] The array of behaviors described serves to stimulate and synchronize the hinds during the phase of transition between the anestrus period of summer and the active breeding season. Hinds may go through a silent heat and both hormonal activity and follicular wave activity increase until an ovulatory cycle occurs.[1] A "dormitory effect" is also recognized to occur among hinds, and silent heat may further

*Note on terminology: Female red deer are called hinds, as are wapiti (North American elk) in some texts. Most industry people call wapiti females cows. The males of the two subspecies are technically both called stags, which is in common use for red deer; the more common term for wapiti is bulls.

synchronize estrus among a group of hinds, thus shortening the rut and calving period.

The intensity of stag behavior increases gradually until the animals are in full-blown rut. The rut is defined as the period of maximum reproductive activity.[2] It is timed to meet the evolutionary pressures that have dictated parturition onset to meet the optimal forage availability in the following year.

Sparring

Sparring encounters, when antlers are engaged and pushing matches occur, are a common feature of the rut in free-ranging animals. They are most intense in mature individuals and are unusual if males are of different ages. However, they are usually preceded by a series of ritualized behaviors during which males size one another up. Stags may break off an encounter before engaging in physical contact.[7]

Vocalization

The calling sound made by rutting males is known as roaring in red deer, and bugling in wapiti. By the onset of the rut mature stags have been calling for at least 2 or 3 weeks. Stags will call whether or not they are placed with hinds. If the weather is cool (<10° C) during the day, calling may occur as often as twice a minute throughout the 24-hour day, but if temperatures exceed 20° C the frequency declines during the middle of the day. The sounds are most commonly heard before 9 AM and after 5 PM.[4,9] In red deer frequency increases to as many as eight roars per minute preceding a fight.[9] In red deer it has been shown that the frequency of roaring may affect the success that a stag has in maintaining his harem.[9] It is rare to hear a yearling wapiti bugle at all.

Heterosexual Behavior

Hinds tend to move into rutting areas held by dominant stags over this period. While they are nonestrous, hinds remain subordinate to the stag, displaying characteristic submissive behaviors whenever approached. These behaviors include a head lowering and neck stretching posture associated with jaw chattering. Hinds will often run short distances to avoid the stag. Stags frequently display to hinds, particularly if they appear to be moving outside the stag's rutting area. Such displays include standing side-on to the hind with chin raised. The stag will often raise and stamp a front foot, emitting a deep guttural bark at the same time. This is usually sufficient to direct the hind back to the core rutting area or to join the other hinds. The stag will frequently wander among resting and grazing hinds, sniffing each hind's perianal region for signs of impending estrus. Hind urine is often tested with displays of flehmen.[6,10]

In natural mating systems wapiti and red deer hinds form groups of several animals and these are "rounded up" by rutting stags. The amount of time spent herding females increases steadily from the time that velvet is cleaned off, and within about 2 weeks has become a major activity.[4] Rutting bulls will spend up to 40% of their time herding their females on farms, even chasing them away from water or feed troughs.[6] Most of the herding behavior is seen in the early morning up to 9 AM or in the evening as during the hottest part of the day stags and hinds may spend up to 60% of their time resting. The threat employed by stags that are herding hinds is usually extension of the head and neck such that the head is level with or slightly below the shoulders. This posture, held while the stag runs toward a hind that is trying to leave the herd, often stimulates the hind to rejoin the group, whereupon herding activity ceases.[4]

Yearling males may not show full rutting behavior for as much as a month later than older ones and may be seen making homosexual mounts, but they are capable of breeding if older males are not present.[2,11,12]

Throughout the rut the stags can be seen periodically checking the hinds, both by smelling their perineal regions or faces and by sniffing their urine and performing "flehmen," or lip curl.[10] The stag may walk among a group of resting hinds carrying out these checks and if a hind is coming into estrus the stag will pay more attention to her, sometimes separating her from the herd.

The hinds show little overt behavior related to reproduction in the period preceding the peak of the rut. Only the most vigilant observer will be able to detect when a hind is in heat. There is little or no vulval swelling, and although it does occur, hind mounting of other hinds is unusual. The well-known research team on the island of Rhum have reported that red deer hinds give off a distinctive odor during the time that they are in estrus.[2] The interaction of a hind with the stag is the best indicator of her reproductive state. Hinds may exhibit different behavior if they are kept separate from stags.[13] They would approach fences separating them from the male, frequently reaching through to lick him, while he became agitated and ran along the fence bugling frequently. Hinds approaching estrus may spend an increasing amount of time paying attention to the stag, or there may almost no interaction between them until the moment of coitus.[13]

The range of behaviors that are engaged in by elk and red deer have been extensively studied by many observers. One of the earliest "textbook" references was published in 1576 and is an account by Turbevile of many aspects of hunting and the biology of deer.[12] A section of his chapter on the "Vault of Hartes"* bears quoting as it cannot be much improved upon today.† The second paragraph quoted contains what is probably the most lyrical description of flehmen ever penned:

> Harts do beginne to Vault about the middeft of September, and their Rut doth continue about two monethes, and the older that they be, the hottere they are, and the better beloued of the Hyndes. The old harts go fooner to vault than the yong, and they are fo fierce and fo proude, that vntil they haue accomplyfhed their luft, the yong Harts

*Hartes (or harts) is the medieval English term for a mature red deer stag.

†Note that in medieval English f (without the full horizontal bar, so it cannot be properly represented on modern keyboards) is often used for the modern s, and u is used to represent v.

Table **127-1**

Time Sequence of Breeding Behavior in Red Deer

Action	Hours before Mating
Sniff-approach by stag	22
Short chase by stag	17
Long chase by stag	6
Low mount	45 min
Mount by hind Copulation	20 min

From Veltman CJ: The mating behavior of red deer. Proceedings of the 2nd Deer Course For Veterinarians, Deer Branch of the NZ Veterinarian Association, 1985, pp 135–142.

Table **127-2**

Breeding Parameters of Wapiti and Red Deer[6,13,14]

Parameter	Measurement
Average low mounts per copulation	4 (1–16)
Duration of low mount	15 sec
Duration of copulation, mount included	5 sec
Repeat breeding (some hinds only)	0.3–7 h
Duration of postcopulatory straining	3.2 min
Time taken by hind to return to herd	9 min
Duration of estrus	6–30 h
Estrus interval	21.2 (19–25) (wap), 18 days
Gestation length	247 ± 5 (wap) days

> dare not come neare them, for if they do, they beate them and dryue them away. The yong Deere haue a maruellous craft and malice, for when they perceiue that the olde Hartes are wearie of the Rut and weakened in force, they runne vppon them, and eyther hurt or kyll them, caufing them to abandon the Rut, and then they remayne maifters in their places.
>
> Hartes doe muche foner kyll each other when there is fcarcitie of Hyndes, for if there be Hyndes plentie, then they feparate them felues in one place or other. It is a pleafure, to beholde them when they goe to Rutte and make their vaute. For when they smell the Hynde, they rayfe their nofe vp nto the ayre, and look aloft, as though they gaue thankes to nature which gaue them fo great delight.

More modern descriptions of breeding behavior differ only in detail from those of Turbevile. Some of them are indicated in Tables 127-1 and 127-2.

Precopulatory Behavior

In free-ranging situations, for about a 12-hour period before estrus the hind and stag may be seen resting near one another, often within 10 or 15 m, and the stag appear to be guarding her.[14] As estrus approaches in a hind, the stag may single the hind out and initiate short (10–20 m) intensive chase sequences, usually with his neck stretched forward and with rapid tongue movements directed toward the hind's rear (Fig. 127-1). Such chases usually end with the hind remaining within 5 to 10 m of the stag. These sequences have been observed to occur anywhere between 6 hours and a few minutes from the onset of estrus (actual receptivity).

Fig. 127-1 Stag and hind in a chase sequence. (From Haigh JC, Hudson RJ: *Farming wapiti and red deer*. St Louis: Mosby, 1993.)

On farms, where mating groups are not as free to move about, this behavior may not be observed, especially if large numbers of hinds are run with a single stag and he may be mating with an average of three or four a day. The stag will, from time to time, approach a hind that is coming into estrus while stretching his head and elevating his tail. The hind may move away and the stag chases her for short distances and then appears to lose interest.[10,14] The chases increase in length as the hind approaches standing estrus and solicitation by hinds may also be seen. They may stop, arch their backs, and elevate their tails. The gait may change to a prancing walk.[10]

Mounting and Copulatory Behavior

Depending upon his level of experience and the number of hinds that he has to breed, a stag may perform so-called low mounts.[6,10,13,14] During these he will mount the hind and lay on her back. His penis protrudes from the sheath, but no intromission occurs. The low mounts may be seen as much as 12 hours before mating, but usually this time is less than 6 hours (Fig. 127-2). In an extreme case 34 low mounts by a single red deer stag were seen.[14]

Hinds in estrus may sometimes mount stags or other hinds and spend a good deal of time rubbing themselves over the rump or under the neck of the stag.[6,10,13,14] There is a considerable difference among individual hinds as to the degree of estrus behavior shown. Some are served only once at a given estrus and may show only the most subtle signs of being in heat. Others may be served four or five times and show conspicuous estrus behavior.[13,14]

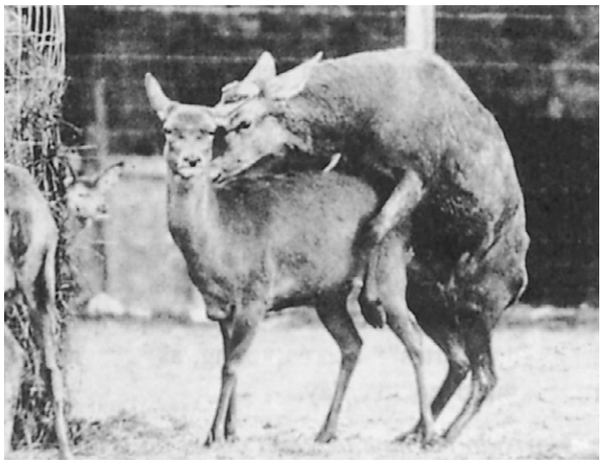

Fig. 127-2 Low mount without intromission. (From Haigh JC, Hudson RJ: *Farming wapiti and red deer*. St Louis: Mosby, 1993.)

Estrus onset is often quite sudden, at which point the hind stops abruptly during a chase sequence and allows the stag to mount. She may even take several steps backward with her tail raised and wagging.[13] When this happens still the stag will show great excitement, his tongue will often flick in and out, and he will arch his neck over the back and neck of the hind.[6,10,13,14] One or two low mounts then usually occur in quick succession, followed by the ejaculatory mount. Prolonged mating sequences, involving many noncopulatory mounts, can occur. Under these circumstances, the total duration of courtship may last several hours and the hinds often exhibit more obvious signs of estrus. Such behaviors include self-grooming by rubbing her chin over her flanks, similarly grooming the stag, mounting or being mounted by other hinds, and mounting the stag. The hind will generally remain very close to the stag until copulation occurs.

The actual moment of copulation and ejaculation lasts only a few seconds. The hind stands after a brief chase and the stag mounts and then thrusts upward in a single motion. He places almost no weight on the hind's back, but the force of the thrust usually drives the hind forward a few paces. This strong physical thrust has been reported to occasionally cause vaginal tearing or rupture, and may be responsible for penile injury in some instances.

There are differing reports on the time of day at which copulation occurs. There do not appear to be any reports that describe continuous observation over a prolonged period of both night and day in natural breeding situations, most studies involving either only daylight hours or specific times of day.[6,10,13,14] Marking of hinds by raddle painted stags occurs throughout the 24-hour day,[6,14] but Veltman has reported that 75% to 90% of hinds were mated during dawn-to-dusk watches.[10] In one study of synchronized hinds continuously observed for 72 hours, using night vision equipment between dusk and dawn, coitus was observed during both night and day.[6]

Postcopulatory Behavior

After dismounting the stag will often roar or bugle before walking away and apparently losing interest. Just after mating the hind will adopt a squatting stance and strain. Both urine and mucoid fluid are passed from the vagina.[4,10,13,14] This posture is not seen at other times.[10] After a few minutes the hind will rejoin the herd. She may breed again with the stag, even after a few hours, and copulation intervals in excess of 24 hours have been recorded.[14] Some hinds terminate estrus at copulation, but 30% to 40% will re-mate 6 to 12 hours later. The average time for a red deer hind to be in estrus is 18 hours. Up to four copulations per estrus have been observed.

Although this pattern of estrous and mating behavior is typical for red deer, there is considerable variation on the theme, particularly in relation to the duration of events and the number of noncopulatory mounts.

The End of the Rut

The rut may last for only a few weeks with a peak activity of as little as 20 days.[4] A resurgence of activity may occur if hinds do not conceive during the first period.[6] In free-ranging situations, if a stag is exhausted during this time he will be usurped by another.[4] As indicated previously and noted by Turbevile, the young stags come into the peak of their rut later than the mature ones.[12]

Rutting stags spend little time feeding, and a mature stag may lose as much as 25% of his body weight in 6 weeks, and losses of 30% have been recorded.[2,4] Bulls or stags that are markedly debilitated after rutting must soon regain some condition in order to survive harsh winters.

HIND BEHAVIOR

Calving/Fawning

The birthing process of red deer and white-tailed deer has been well described, based on behavioral observations on parturient females. The process is similar to many other ruminant species. However, some key features are relevant to cervid species. Even in gregarious species (e.g., red deer, fallow deer), parturient females seek isolation from herd mates for the birth process. In farmed red deer, preparturient females are often observed to frantically pace fence lines in an effort to isolate themselves from other hinds. The frequency of "fence pacing" decreases with increased paddock area and decreased stocking density, indicating that the behavior is strongly motivated by a desire to seek an isolated birthing environment. Such behavior is observed anywhere up to 48 hours before parturition, but is intensified in the 24 hours leading up to birth.

Actual parturition is similar to that seen in other domestic and wild ruminants. The process from initiation of labor (i.e., abdominal straining) to expulsion of the calf/placenta can occur over periods of 30 minutes to 6 to 8 hours, but normally takes 2 to 3 hours. Considerable variation in the timing of these events has been observed in wapiti.[15] All remnants of afterbirth are ingested by the females.

Posterior presentation is not considered as an abnormality in *Cervus* to the same extent as it is in other domestic species. It has been observed to proceed to normal unassisted birth in a high proportion of cases,[6] Details on timing of calving events are provided.[15]

Placentophagia

It is common to see hinds turning back and ingesting any part of the placental membranes that they can reach, as well as licking and chewing any fluids or tissues that have fallen to the ground, even before calf delivery (Figs. 127-3 to 127-5). Placentopahgia is a normal event in mammalian life, and it has been suggested that it is related to several factors. The commonest is that it is a predator defense mechanism. Other, unsubstantiated, reasons suggested include that this tissue may act in aiding milk let-down, or may provide a substantial supply of nutrients after a period of reduced intake.[16] The most detailed explanation comes from work in rats, in which it has been shown that the fetal fluids and placenta contain placental opioid-enhancing factor, a protein that enhances endogenous opioid-mediated analgesia evident at the end of pregnancy and during delivery.[16,17]

Bonding

Usually, as soon as the calf is born the mother starts to attend to it, especially licking and drying it and eating the remains of the afterbirth, even if this is expelled some time after the actual delivery, but the hind may rest if parturition has been prolonged.[1]

Free-ranging deer tend to leave the herd and deliver their calves in isolation, only rejoining the herd after a period of several days once they have bonded with the calf. In farm situations this option is not available to them, and this may explain the observed occurrence of cross-mothering that is recognized in red deer and wapiti.[6]

In farm situations a dominant hind experiencing second stage labor may find a newborn calf hiding in the paddock and adopt it. When her own calf is born some time later she may abandon one of the two, or her own calf may be taken by the subordinate hind that has been driven off and is still seeking a juvenile to raise.[18]

Lactation

Most species of deer nurse their newborn calves for a few hours after birth, during which time dam/calf bonding occurs. This may sometimes take place when the hind is lying down (Fig. 127-6). Thereafter, the dam leaves the calf hidden at or near the birth site, returning only a few times per day during the first 2 to 3 weeks to suckle the calf. Calves follow their dams, often returning to the herd, after this initial "hide-out" period. Anecdotal evidence suggests that in farm situations the hide-out period

Fig. 127-3 Wapiti hind cleaning membranes and fluids during early parturition. (Courtesy of Jerry C. Haigh.)

Fig. 127-4 Wapiti hind reaching back to consume fetal membranes shortly after parturition as the calf stands and begins to seek the udder. (Courtesy of Jerry C. Haigh.)

Fig. 127-5 Wapiti hind consuming remains of expelled placenta. (Courtesy of Jerry C. Haigh.)

Fig. 127-6 Wapiti hind and calf lying down soon after parturition. The hind cleans or stimulates the anus of the calf while the calf sucks. (Courtesy of Jerry C. Haigh.)

may be shorter in later born calves within a single paddock. Exceptions to this general pattern do occur among cervids; reindeer and caribou calves generally follow their dams within a few hours of birth, owing to the migrating and wide-ranging nature of this species.

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CHAPTER 128

Reproductive Management of Farmed Red Deer and Wapiti

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Currently, the various races or subspecies of red deer, and the wapiti (or North American elk), which is found in eastern Asia as well as North America, are all considered to belong to one species, *Cervus elaphus*.

The historical range of red deer stretches from Scotland to China, with perhaps as many as 22 subspecies recognized.[1] Red deer have been introduced into the wild in numerous countries. They have been domesticated as a livestock species in many countries, but particularly in New Zealand, where approximately 2.3 million animals were farmed in the year 2001.

Wapiti, of which at least 10 subspecies are recognized, range, in Asia, from the Tien Shan mountains of Russian Turkestan and the Altai mountains of Russia and Mongolia through China and Manchuria into North Korea.[1] In North America they were formerly found throughout much of the continent. Their range was reduced through overhunting in the late 19th century, but numerous reintroductions have occurred since. They are now found, both free-ranging and on ranches and farms, in many areas.[1]

Reproductive management of *C. elaphus* varies somewhat according to the region or country in which these animals are farmed and is influenced both by market forces and by climate. The numerous subspecies can interbreed freely. For this discussion, crosses between them are referred to as crossbreds, rather than the more commonly used, but technically incorrect term, hybrids.[1] One anomaly discussed in more detail in these pages is that red deer and wapiti respond differently to ovarian superstimulation.[1]

Fundamentally, the species is described as seasonally polyestrous, or a short-day breeder, with the onset of fertility and the rut occurring in autumn. The timing of onset of the breeding season has evolved by the need to deliver calves at a time when grazing conditions are optimum for milk production and calf well–being, in early summer. Gestation of wapiti is slightly longer (247 ± approx. 5 days) than that of red deer (233 ± approx. 7 days).[1,2] For this reason wapiti stags clean the velvet antler (an event closely linked to elevated testicular testosterone concentrations) and display aggressive behavior prior to the rut, about 2 weeks earlier than red deer.[1]

Use of the larger wapiti stags, or their F_1 cross red deer male progeny, as terminal sires (those whose progeny are destined for venison production) over the smaller red deer hind developed in New Zealand to increase the biologic efficiency of the production system. Most red deer hinds weigh 90 to 120 kg, whereas mature wapiti hinds weigh 250 to 300 kg. Progeny growth rate increase is related to the proportion of wapiti genes present. Because the progeny grow fast, more venison can be supplied to meet the seasonal Northern Hemisphere market.

In New Zealand management is also designed to bring forward the median herd calving date as much as possible to take advantage of early spring grass growth.

BODY CONDITION AND BODY CONDITION SCORING

Body condition score (BCS) is an important tool for management of dairy and beef cattle. BCS systems have been developed for both red deer and wapiti.[3–5] Scoring charts have been developed with scores ranging from 1 (lean, or very poor condition) to 5 (fat, or very good condition) with half-unit increments[3,5] or from 1 (thin or emaciated) to 9 (fat or obese).[4] For the purposes of the following discussion the 5-point scale of Audigé and associates[3] is used. It is essential that palpation be employed for accurate estimation of condition, particularly in winter when coat obscures the body contour. As the winter coat is usually well developed by the onset of the rut, the time when a BCS is most relevant, care must be taken to avoid missing subtle changes.[6,7] Eye appraisal of animals in the paddock may estimate herd status to within one score, but is inadequate for individual animal condition scoring.

In the most objective study reported, in which deer were palpated,[3] showed repeatability was 0.91 and 0.89, while reproducibility was 0.81 and 0.79 for yearlings (primiparous) and adult hinds, respectively. There were significant variations among hinds according to season and farm.

Although the only data on BCS in deer have related to reproductive outcomes, it is likely that health and mortality rate are also related to body condition, since low BCS is an indicator of nutritional status.

Stags

Males should enter the rut with a BCS as close as possible to 5 because they will lose from 25% to 30% of their pre-rut weight, even if offered grain and good quality hay, often resulting in a BCS of 1.5 or 2 in the early winter. In mature stags this change is seen even if the stags do not have access to hinds, as they spend considerable amounts

of time engaging in rutting behavior. Yearling stags (18 months of age) do not lose appreciable weight or BCS during this period, and 2- and 3-year-old stags lose about 15% of body weight.

Stags require a high plane of nutrition immediately after the rut to recover adequate condition before winter, to reduce the risk of death due to exposure. Grain or concentrates or high pasture allowances should be provided for at least 6 weeks before the winter solstice. By this time, animal and human safety is usually not a problem, as aggressive behavior has subsided. A useful target by the winter solstice is a recovery of 25% to 30% of the body weight lost, or a BCS increase of 1 point. Gradual increase in grain allowance avoids grain overload and laminitis, to which deer are particularly susceptible.[1]

Hinds

Body Condition Score and Conception

In red deer, premating BCS is significantly associated with conception rate, conception date, and weight of weaned offspring, while BCS during pregnancy is associated with dystocia and ability of the red deer hind to rear a calf to weaning.[3,8]

Guidelines for achievement of optimal reproductive performance have been proposed. In general, hinds with a BCS of 2.5 or more have a greater chance of conception per se, and have an earlier conception date. Pregnancy rates of hinds in good condition (3–5) are likely to be 10% greater than hinds in poor condition (<2.5).[9] However, it has been observed that overfat primiparous hinds (>BCS 4) were at increased risk of failure to conceive.[6,7,10] However, Beatson and associates[11] were unable to verify this relationship.

If the production target is "optimal" reproductive success, defined by an early mean conception date and high conception rate, individual hind BCS should be greater than 2.5 before mating and should either be stable or increased during the mating period.[6,7] Indeed, Audigé and associates[3] propose that for optimal reproductive performance from hinds, their body condition should remain stable throughout the year at scores between 2.5 and 4. Recent studies have shown that a delay in mean conception date of 12 days was associated with late weaning, resulting in an average loss of 0.5 BCS.[12] The pattern of change during the mating period is also important. Beatson and associates[9,11] showed that hinds below a BCS of 2.5 before mating, but increasing BCS during the mating season, have a higher conception rate than those staying below 2. Those at or above BCS 2.5 that gained in BCS during mating had no improvement in conception date or rate, confirming that BCS 2.5 or greater is optimal. However, these authors demonstrated that hinds losing BCS from any premating score showed a lowered conception rate. These observations confirm the value of BCS as a predictor of reproduction outcomes, and as a tool for determining when management changes are appropriate if optimal reproductive performance is desired.

Similar criteria for wapiti have not yet been verified, but the principles are likely to be the same. Although 2-year-old wapiti hinds (jinnocks) calved (in the Northern Hemisphere) later than adult hinds (mean 11 June versus 6 June), hind pre-rut weight influenced calving dates.[13] This finding confirms that there is an interaction between BCS and body weight, particularly in primiparous hinds.

Body Condition Score and Dystocia

It has been generally believed that the fat, unfit hind is more likely to suffer dystocia than one that is physically fit and in optimal body condition.[1] Recent research has shown associations between BCS and dystocia in red deer herds.[8] Hinds with a BCS of 4 or more shortly before calving were 2.7 times as likely to experience dystocia than hinds with lower scores. An increase in BCS late in gestation also increased the risk of dystocia. There was an interaction between BCS and topography, with hinds with a high BCS but grazed on steep hill pastures being at lower risk of dystocia than hinds of the same BCS grazing flat pastures.

GENETIC SELECTION

Selection criteria for red deer have been developed, to some extent, in New Zealand. For practical purposes, the selection criteria applied by most deer farmers are quite basic. For hinds and stags, temperament is a prime determinant. This is judged by ease of handling for routine procedures such as yarding, vaccination, parasite control, ultrasound scanning, and for stags, velvet antler removal. An important consideration is aggression, both toward handlers and toward other deer. The heritability of temperament not known, and traits may be learned rather than genetic. However, farmers report a significant improvement in achieving calm, amenable deer as a result of culling animals that do not confirm to requirements. Selection of hinds for ability to conceive, as shown by ultrasound scanning early in gestation, and rear a calf to weaning, determined by lactational status, is also widely practiced. Audigé and associates[6] demonstrated that hinds that reared a calf to weaning were 3.1 times as likely to conceive early, and 3.7 times as likely to conceive at all in the subsequent breeding season than hinds not rearing a calf to weaning. Some farmers using individual sire mating systems will select stags on the basis of their apparent fertility, measured by conceptions or calves born.

Body size is also a selection criterion, and many farmers use crossbreeding between red deer and wapiti to achieve increased size compared with purebred red deer. However, this may reduce the biologic efficiency of feed conversion to venison, because hind maintenance constitutes a higher proportion of total feed requirement for the combined mother-offspring unit. Alternatively, use of wapiti/red deer hybrid stags mated to pure red deer hinds improves the biologic efficiency of a venison production system because of the greater potential for growth of the crossbred offspring, thus increasing the proportion of total energy consumption of the mother-offspring unit partitioned to venison production.

Selection of sire stags for velvet antler production is commonly practiced, and this has the potential for significant improvement in antler production because the heritability is about 0.43. Currently, phenotypic

characteristics of weight and conformation are the main criteria. Calculation of breeding values based on individual animal recording is only recently being adopted by some deer stud farmers in New Zealand. Sire referencing now needs to be undertaken to improve the rate of genetic gain.

No such criteria exist for wapiti. Until subspecies, races, and bloodlines have been sufficiently studied to establish true genetic differences, there is little basis for designing complex breeding programs. Until referencing schemes, such as ones initiated in New Zealand, have been developed, selection will inevitably be based upon phenotypic characteristics.

Hinds may be selected for temperament, body size, and appearance. These selection criteria need not have any basis in genetic performance, which can only be judged from progeny. However, some of these have been put to use. Culling has been based on infertility, repeated difficulty with calving, poor temperament, or other undesirable features as criteria.

In North America the driving force behind the wapiti industry has been the single characteristic of antler size, without regard to pharmaceutical quality. Some differentiation between so-called "velvet" character and "hard antler" character has begun to develop. The former products are used in the neutraceutical trade in the Orient, and to an increasing extent in North America. Hard antler (also known, inaccurately, in the trade as "hard horn") size and length is the character sought in the hunt ranch market. Stags may also be selected for bloodline and relationship to hinds in a group as well as phenotypic characteristics such as size, appearance, and temperament.

HERITABILITY

There have been a few studies of heritability in red deer in New Zealand and the United Kingdom. Where stag herds are farmed for velvet antler production, producers are interested in selection of stags for superior lifetime performance and selection of sires and dams to produce improved sons and management of the stag herd to increase returns from velvet antler.[14] These authors found that the heritability of antler production studied among over 2000 stags on five farms ranged from 0.43 (±0.09 SE) to 0.85 (±0.33 SE). The average estimated genetic correlation between velvet weights in successive years was 0.97 (±0.07 SE) but declined to 0.76 (±0.29 SE) as the number of years between harvests increased.[14]

Live weights have moderate to high heritabilities in both sexes and at all ages with estimates ranging from 0.48 (±0.36) to 0.80 (±0.12)[14] and 0.31 to 0.49, 0.22 to 0.89, 0.33 to 0.48, 0.37 to 0.45, and 0.37 to 0.90 for birth weight, weaning weight, midwinter weight, turn-out weight, and other weights, respectively.[15] On one property where inbreeding was recognized as a factor, heritability estimates were very low (<0.08).[15] Heritabilities for date of calving were low on seven of the eight farms (<0.05), and repeatabilities were low to moderate (0.06 to 0.37).[15]

Animals whose bloodlines originated in the forests of Eastern Europe (Yugoslavia, Hungary, Germany) were heavier at all stages, indicating their usefulness as "terminal sire" breeds, confirming the potential usefulness of the larger mainland European red deer.[15] Hinds of mainland European parentage also tended to calve earlier.[15]

There is only limited information on heritability of production traits in wapiti. One study of heritability of antler traits in over 12,700 stags showed a moderately heritable figure of 0.27 (±0.03 SE).[16] Antler yield can therefore be expected to respond to selection to some degree, but nonadditive and environmental effects can also influence production.

Crossbreeding involving mating animals of different breeds or races is prohibited in some jurisdictions (e.g., Alberta and Manitoba, Canada, and several states in the United States).

MATING MANAGEMENT

Decisions about mating management determine the likely pattern of reproductive outcomes. One of the most important considerations in deciding a breeding strategy is the choice made by the farmer as to how long a calving season is tolerable, and therefore at what date the stag is withdrawn from the hinds. As productivity and growth rates of calves is governed by birth dates, the earlier that hinds deliver, the higher are progeny weights. For most practical purposes a 6- or 8-week calving season is acceptable, although on well-managed properties most of the calves delivered in the second month will be born to 2-year-old animals calving for the first time.

To achieve the maximum benefit of the "stag effect," in which the sight, sound, and smell of the stag will both synchronize and stimulate the onset of estrus in hinds, it is advisable to introduce a breeding male to a hind mob 2 or 3 weeks before the expected date of breeding. In practical terms this is best accomplished by introduction late August, and before the end of the first week in September in the Northern Hemisphere.

Although some producers do request a prebreeding sire evaluation, the semen testing of stags just before the rut is probably not a common practice, despite the fact that infertility in stags is recognized. *Brucella ovis* has become recognized in New Zealand as a pathogen that affects the reproductive system of stags and is capable of causing infertility.[17]

Stag/Hind Ratio

The mating capacity of stags is not well documented but is an important consideration in determining pregnancy dates and herd conception patterns. The average stag/hind ratios for adult and yearling red deer in New Zealand were 1:40 (range 8–82) and 1:27 (range 1–51), respectively. The most recent data has shown pregnancy rates and dates were similar for more than 80 groups of red deer hinds mated at single sire ratios as low as 1:140.[11,18] Thus, the mating capacity of stags is greater than commonly utilized. It is recommended that all management factors for achieving pregnancy, as described elsewhere,[6] be optimal when large numbers of hinds are mated to an individual stag. There are obvious advantages to the use of large mating groups. The number of

paddocks needed is reduced, more hinds can be mated to superior sires, and the number of breeding stags required is reduced. However, as Beatson and associates[9] suggest, the use of large breeding mobs increases the risk of financial loss if, in a single sire mating system, a stag is either infertile or subfertile. Stag failures do occur, regardless of breeding group size, so the use of back-up stags should be standard practice if the risk is to be reduced.[1,9] There are no data to indicate a usable maximum number of hinds per stag on wapiti farms.

Despite the observation of a greater mating capacity of stags than commonly believed, it has become common practice to manage breeding groups with about one mature stag to 60 red deer hinds[6] and about a maximum of 35 or 40 wapiti hinds. In the most extensive investigation to date, over 3600 red deer hinds over 2 years of age, and more than 1100 yearling hinds were mated in each of 3 successive years. The number of hinds per stag was classified as 0–60, 61–100, and above 100. The variations in calving dates about a median were 10.0, 10.3, and 11.3 days, respectively.[9]

In general, much smaller numbers of hinds are mated to yearling stags if they are used as single sires. Groups of less than 10 hinds per stag are commonly used, although no data are available to justify this practice. In New Zealand it is not uncommon to breed yearling hinds in mobs that include several yearling males.

Manipulation of the Natural Cycle

The median calving date in New Zealand averages November 30 (range 11/26 to 12/6) for adult hinds, and December 13 (range 11/26 to 12/15) for primiparous hinds. In many temperate climates this is an ideal situation that ensures that the best possible quality of feed is available to the hinds to meet the demands of lactation. In some parts of the world, particularly New Zealand, the best quality forage is available slightly earlier in the year, and research efforts have been directed to finding ways of inducing early reproduction so that hinds will calve when forage is at its best.[1] In such areas it has the added advantage that calves born early in the year may attain heavier weights at a set time, and potentially provide better returns at market.

The technique has been investigated in New Zealand, but in North America, it is only likely to be of use in the warm wet climatic regions of the west coast and more southern areas where good quality fodder is available for long periods of the year. This may also be true of areas where irrigated grazing is practiced.

There are a number of practical methods of inducing early estrus in deer herds. The most commonly used regimen is a combination of a controlled intravaginal drug (progesterone)-releasing (CIDR) source for about 12 days, followed by intramuscular injection with 200 IU pregnant mare serum gonadotropin (PMSG) or synthetic gonadotropin-releasing hormone (GnRH) at the time of CIDR withdrawal. This regimen is also used for estrus synchronization for artificial insemination (AI) and embryonic transfer (ET) programs. An additional observation is that hinds in contact with treated animals are induced to come into estrus early, presumably due to a "dormitory effect" of the type that has been reported in other animals, including humans. The "stag effect," achieved by early introduction of stags to hind groups prior to the mating season, is also known to be a powerful inducer of estrus in hinds and one that can be enhanced further by advancement of rutting behavior of stags using melatonin. Advancement of the median conception dates of 8 to 10 days has been achieved using this technique. The sight, sound, and smell of the stag all serve to stimulate reproductive activity in the females.

If significant advancement of estrus is induced in hinds, similar advancement of stag reproductive activity is necessary. In natural systems, the cycle of sperm production among stags is geared to a calendar that more or less matches that of the hinds coming into estrus. The best quality semen is only present about 2 weeks before the rut, as it takes around 60 days for the testicle to go from its quiescent state around the longest day of the year to the production of fully mature spermatozoa. If hinds are artificially induced into estrus very early, the stags may be subfertile. Melatonin treatment of stags can resolve this problem. Alternatively, artificial insemination using frozen semen can be undertaken.

The other method for estrus advancement that has proved successful is use of melatonin. The advantage of melatonin is that it can be used upon both hinds and stags, either in the feed or as implants, so that both sexes come into reproductive readiness together. A melatonin implant has been approved for commercial use in Australia and New Zealand. It is recommended for use in yearling hinds in the first year. It is not commonly used with adult hinds because they are calving at the time when administration is needed. If administered to pregnant hinds it may cause prolonged pregnancy. The recommended regimen involves monthly treatments starting 3 to 4 weeks before the longest day. This will advance the reproductive season by approximately 3 to 6 weeks. Treatments started later than about 10 days before the longest day may advance calving by approximately 2 to 3 weeks. Treatments earlier than recommended are contraindicated and may suppress the ability of both stags and hinds to respond. Only those stags that are to be used in early breeding programs should be treated, as their performance if used later during the regular season may be less than optimal.

Melatonin induces not only early reproductive activity but also visible effects, including an early molt to winter coat, a reduced milk yield in the hinds, and an earlier than usual decline in appetite.

Pregnancy Determination

Details of techniques for pregnancy determination of both cervids and bison are provided in a separate chapter.[19–21] They include manual palpation, ultrasound scanning, and methods involving blood or fecal sampling.

Pregnancy Profiling

One advanced application of ultrasound to pregnancy diagnosis and assessment of fetal aging as a tool for

measuring reproductive performance has been described as pregnancy profiling.[18] Data have demonstrated the range of reproductive patterns that provide a benchmark for farmers wishing to improve reproductive performance. Pregnancy profiles for individual deer herds are becoming common on New Zealand deer farms, allowing managers to define not only pregnancy rate but also other reproductive outcomes such as first and last expected calving dates, median and mean conception dates, and the pattern of conceptions. Management decisions, as discussed elsewhere in this chapter, can be targeted to achieve the desired outcome.

PARTURITION

A distended abdomen is one of the few visual clues of impending parturition in red deer and wapiti.[1] In adult hinds the udder may begin to enlarge as much as 4 weeks before calving, while in primiparous hinds, this development may not be seen until 2 weeks prior to parturition.[1]

Restlessness, a characteristic fence-pacing activity, and solitude seeking are characteristic of imminent parturition. New Zealand workers recognized two such behaviors.[1] This behavior is not always seen, and may, in part, be a herd density–dependent phenomenon. In the wild, hinds usually isolate themselves as parturition approaches and do not rejoin the herd until about 2 weeks after calving.[22] Fence pacing appears to be an attempt to find a more suitable location for calving or to get away from pen mates. As few as 30% of farmed wapiti exhibit fence-pacing activity if they are at a density of less than three hinds per acre and have some measure of isolation from one another through either topography or the presence of cover.[23] However, age and experience of the hind does not seem to be a factor, and those that fence pace are not necessarily the same individuals each year.

By the end of first-stage labor the parturient hind will usually separate from the herd. As labor advances she may look at her flanks and frequently change position. She may even start to strain, stop and move away, and then graze briefly before starting again. Close observation of the events at calving have demonstrated that there is considerable variation in timing (Table 128-1).[24]

The normal fetal delivery presentation is anterior dorsal, similar to that of cattle, with the forefeet coming first, followed by the head.[1] However, a posterior presentation is not uncommon. During parturition the hind will turn and lick herself or the emerging calf or ingest fetal fluids that have splashed on the ground, as well as the placenta.[1] In normal parturition, passing the calf's head through the pelvis is the rate-limiting step, with complete delivery usually occurring in a matter of seconds thereafter. Final delivery may take place when the dam is either lying or standing.

Disturbance of a hind during parturition may delay the process considerably, and particularly in primiparous hinds, may predispose to dystocia. Hinds may rejoin the herd and subsequently seek a new calving site. Furthermore, primiparous hinds may leave the calving site altogether and abandon the calf if disturbed.[1]

Table **128-1**

Calving Sequence and Calf Vitality in Farmed Wapiti Observed at the Ministik Wildlife Research Station from 1988 to 1993

Observations	*n*	Range	Mean ± SEM
Stages of Parturition		**Time before Delivery (min)**	
First stage			
Pacing	27	91–902	365 ± 38
Second stage			
Contractions first seen	31	15–720	122 ± 24
Appearance of amniotic sac	12	27–238	97 ± 22
Bursting of amniotic sac	30	<1–355	79 ± 17
Appearance of calf	55	<1–193	45 ± 5
Calf Development		**Time after Birth (min)**	
Third stage			
First stand	58	13–271	49 ± 5
First suckle	58	28–223	76 ± 5

From Church JS, Hudson RJ: Calving behaviour of farmed wapiti (*Cervus elaphus*). *Appl Anim Behav Sci* 1996;46:263–270.

Calving Dates

The peak of the calving season in the wild and on game farms is the first of June (12/1 in Southern Hemisphere), although variations from year to year and from place to place may occur. At least in wapiti, male calves tend to be born several days later than female calves but this might be due to the slightly greater proportion of male calves born to first-time calvers. This seems contrary to the sex ratio bias on the Isle of Rhum, where sons predominate among older dominant hinds.[22]

Most deer farmers find it advantageous to have calving synchrony. Although breeding seasons can be synchronized or even significantly advanced by hormonal treatments, the simplest way to achieve synchrony is by optimal mating management, including maintaining optimal BCS, early weaning, early stag introduction, early removal of the stag, and culling of late-breeding hinds. For optimal calving spread, stag withdrawal should be by November (May). However, for red deer farming in New Zealand, normal practice shows a mean mating termination date of May 10 (Southern Hemisphere).[6] Those authors showed a median calving date of November 30 for adult hinds and December 13 for primiparous hinds, but considerable variation exists among farms.[6] These data confirm that few farmers achieve the biologic potential reproductive performance from their deer.

Birth Weights

Management of hind weight during gestation has a direct effect upon birth and weaning weights of wapiti. Thorne and associates[25] observed that when hinds lost more than 3% of body weight during the second and third trimesters, calf weights were below 16 kg.

Birth weights average about 9 kg in red deer and 18 kg in wapiti, with crossbred calves being intermediate,

dependent on the proportion of wapiti genes present. Male calves tend to be larger than females, as are calves from older hinds. Birth weight is a good predictor of neonatal survival and weight at weaning. Wapiti calves less than 11.4 kg at birth have a less than 50% chance of survival.[25] A similar relationship has been shown for fallow deer.

If hinds are fed to excess during pregnancy, calving difficulties may ensue, due to both oversize of calves and overfat hinds. Underweight calves, usually due to hind malnutrition, should be the exception on farms, although extremes in the wild are evident. In the wild there is often ample opportunity for the hind to regain condition between the flush of vegetation in mid-April and calving in late May or early June. Consequently, it appears safe, if not desirable, to feed to maintenance or even allow a 5% to 10% weight loss of pregnant hinds through the winter feeding period before they are turned out onto spring pasture. However, a lowered weaning rate has been described in red deer if body weight was lost during gestation.[28]

Bonding

Deer calves are "hider" animals and may be separated from the mother for long periods of time. During a 24-hour period the calf may only spend a few minutes with its mother, during four to seven sucking bouts that last a short time. It is therefore important for both animals to establish recognition of one another. Bonding is initiated when the hind first licks the calf after birth, but is reinforced on subsequent occasions. After a feeding bout the hind attempts to lead the calf away with repeated vocalizations and nose-to-nose contact. At some point the calf will leave its mother and seek cover. After grazing, the hind may return to the calf and initiate contact with a soft mewing. Alternatively, the calf may squeak and even stand up when seeking its mother. If it does this, several hinds may approach and sniff it to identify it. It is possible that a hind may have "labeled" her calf with saliva so that she can recognize it later.[1] However, on farms it is not uncommon to find that a significant number of calves are nursed by females other that their mothers, and that a considerable amount of cross-mothering occurs.[29] Hinds that have lost calves may be willing to let other calves suck, and may even "adopt" a calf.[1]

An on-farm survey to determine the reproductive performance of farmed wapiti among 50 producers in Alberta involving 1084 wapiti hinds reported a calving rate of 81% and 96% for 2-year-old and adult hinds joined with the stag, respectively, giving an average of 93%.[13] Overall calf mortality rate was 5.2% in the survey, with a trend for 2-year-olds to experiencing losses of 9.5% and adult hinds 4.4% of progeny born. Similar surveys of farmed red deer in New Zealand observed calf mortality rates between birth and weaning of 6% to 8% for adult and 12% to 16% for primiparous hinds.[9,28] Dystocia accounted for two thirds of the total calf deaths reported in the survey,[13] but that cause was significantly lower for red deer in New Zealand.[8]

Twinning

Twinning in red deer and wapiti is not common. One set of triplets and three sets of twins gave a multiple birth ratio of 1 in 271 calvings in a Canadian survey of wapiti, while no instance of twinning was confirmed in a New Zealand red deer survey.[13,28] Other data for red deer suggest a twinning rate of between 1:600 and 1:2000. Twinning is common for some other species of deer, including white-tailed deer.

Management at Calving

Prospective dams should be assembled in calving paddocks several weeks before the expected normal calving season to allow time to adapt to the environment and social changes often associated with altering mob composition in preparation for calving. For wapiti in western Canada, an appropriate date is about 10 May. The paddock should provide some cover for the newborn calves but remain sufficiently open and heavily stocked so that the dams can detect and drive out intruding coyotes. Adequate cover near the middle of the paddock keeps calves from bedding along the fence line where they may get tangled in the netting or encounter predators. If the calving paddock is large, strips may be mowed to ensure that vehicles do not drive over hiding calves. Most farmers like to hold their nursery herd near the farmhouse so they can more closely monitor progress and respond to any emergency.[1] However, other studies of red deer suggest that disturbance during calving may increase the risk of "mismothering" and calf loss.

When red deer were first established on farms in New Zealand, losses of calves due to aggression from hinds other than their dams (termed "rogue hinds") were believed significant. Losses were reduced by culling or providing cover (e.g., brush piles or hay coils), but the problem subsided as the breeding herd settled into farm life. Many farmers provide artificial cover in uniform, heavily grazed calving paddocks for thermal amelioration as well as security.

Pasture rotation is advisable to provide the best quality forage available. However, to avoid leaving a newborn behind, the nursery herd should not be moved until the calves have abandoned their lying-out behavior and joined the herd. Supplemental feeds usually are offered to maintain tractability and integrate the calves into the farming operation.

Calving Difficulties

The most common causes of dystocia are overfatness and unfitness.[8] Audigé and associates described higher dystocia rates in hinds with a BCS of 4 or more on flat pastures than on hill pastures. Fat hinds have reduced pelvic passage dimensions and may also bear larger calves, both exacerbating the situation. There has been natural selection for ease of calving because farmed deer have only recently been adapted from feral populations; thus, dystocia rates are lower than in some other classes of domesticated livestock. However, dystocias can be a problem in some poorly managed intensively farmed herds. Occa-

sional incidents of dystocia caused by malpresentations that are not related to mismanagement can occur. Data show dystocia rates on commercial deer farms to average less than 1%, although rates of up to 10% have been observed.[8,26,27] Primary risk factors were high BCS prior to calving, rapid weight gain prior to calving, and topography.

Animals that are timid or are kept in large paddocks, particularly on hills, where they get ample exercise, seldom experience calving difficulties. But the problem may be seen in settled herds that are content to laze around feed bunks throughout pregnancy.[1] Farmers with docile hinds on supplemental feed in the early spring period often encourage animals to be more active in search of emerging spring growth, or force them to exercise to reduce the risk of dystocia. The dystocia rate is higher in primiparous hinds, due to both size factors and their more insecure nature at that age, making them more prone to disturbance during parturition.

Dystocia may also be caused by either fetal or maternal abnormalities. Fetal abnormalities may include congenital conditions but are most commonly either relative fetal oversize or abnormalities of presentation or position as delivery commences. Many dystocias are associated with fetal malpresentation.[8,26,27] Dystocia may be attributable to relative maternal undersize, which may occur if wapiti stags are bred to red deer hinds, or particularly if purebred wapiti embryos are implanted into red deer hinds.

Excessive or inappropriate interference with calving hinds can actually create difficulties. Farmers who enter paddocks where hinds are in second stage labor may disrupt the process and cause hinds to stop and move from the intended birthing site. This is particularly important with primiparous hinds and can cause calf abandonment. The most useful pieces of equipment during the calving season are a vehicle with which the stock are familiar and a good spotting scope or pair of binoculars. With these, paddocks can be observed from a distance. Observation three or four times a day is probably sufficient to detect dystocias in time to allow successful intervention if necessary. However, it is common practice on large commercial deer farms for managers not to observe hinds intensively during calving, because the risk of dystocia in well-managed herds is low, and the risk of disturbance causing dystocia and mismothering problems is high.

A key decision is when to intervene in difficult births. The farmer should be familiar with the chain of behavioral events that occur in normal parturition. If an individual starts the straining characteristic of second stage labor, she should calve within 2 hours if she has had a calf previously, and within 3 hours if she is calving for the first time. A good rule of thumb is that if there is no progress over a 30-minute period, intervention should be considered. The straining may be intermittent, but delay of delivery beyond these times may mean that assistance is required. However, this is not a hard rule, and it is not uncommon to start preparation of the handling shed, only to return half an hour later to find a newborn calf on the ground.

With binoculars, it may be possible to discern whether or not a calf is being normally presented. The normal position, as for most classes of livestock, is an anterior presentation in which the head is preceded by two forefeet. Occasionally, a posterior presentation with only two hind feet showing, may proceed to an unassisted birth. Other presentations should be considered abnormal and intervention should proceed before the usual 2-hour waiting period.

The hind experiencing difficulty should be herded into the handling yards. Although she will most probably have isolated herself from other animals in the calving paddock, and can often be moved on her own, other hinds may move into the yards with her. These animals should be returned to the calving paddock as soon as possible after the parturient dam has been isolated.

It is common for hinds experiencing dystocia to isolate themselves from the herd. She should be yarded and maneuvered so that she can safely be approached initially from the side to avoid being kicked while she is examined. Manual restraint may be adequate, although modern hydraulic restraining devices are excellent in providing both restraint and standing support and are safer for the people involved, particularly with the larger wapiti. Thorough disinfection of the entire perineal area, as well as the hands and arms, and use of copious quantities of obstetric lubricant are essential before any examination of the reproductive tract. If there is any possibility of brucellosis or leptospirosis or other zoonotic disease in the herd, the operator should wear protective gloves and sleeves. The first step is thorough examination of the calf and the hind to ensure that the tract has not been damaged or that the head and feet presented are all from the same calf, or that one forefoot and one hind foot are not being simultaneously presented. Note that twinning in red and wapiti deer is uncommon, but when it occurs, the risk of dystocia is higher. Epidural analgesia and uterine relaxant drugs may be advantageous if manipulations are restricted by abdominal straining or uterine contractions, which may be particularly intense in deer.

Once positional abnormalities have been corrected and it has been determined that the relative sizes of dam and calf will permit vaginal delivery, traction can be applied. This should neither be continuous nor overzealous. Calving chains placed above the fetlock joint of each leg, and if possible behind the ears and through the mouth, the latter to prevent strangulation and ensure that the head is pulled in a straight line, should be pulled both outward and downward. Intermittent traction on alternate legs with steadier traction on the head will usually effect delivery. Overzealous use of chains or ropes can easily lead to limb fractures or uterine rupture, especially among inexperienced operators who are more used to assisting the more robust bovine calves at delivery.

The calf should be quickly removed to an area close to the chute where fresh dry bedding has been prepared. Other than clearing its airways and ensuring that it is breathing, the operator should not interfere with it. Breathing can be stimulated by inserting a finger, or a

short piece of straw, gently into the nostril of the calf to stimulate sneezing. If necessary, intravenous or intracardiac cardiopulmonary stimulant drug may be used. Drying with cloths is contraindicated. Cleaning of the calf should be left to the mother, to assist with calf recognition and bonding.[1] A segment of fetal membrane should be placed on the newborn. If later observation shows the membrane to be still present, this is an indication that bonding or acceptance has not occurred. The hind should be quietly moved to the segment of the handling system where the calf has been left and the two should be left strictly alone for an hour to ensure "mothering up." Rarely, an inexperienced hind may attack a calf, so a brief period of observation of the pair should be carried out. Such an attack would normally take place within a short time (<1 minute).

If immobilizing drugs have been used to restrain the dam, both calf and dam should be treated with an appropriate antagonist, as immobilizing drugs will cross the placental barrier and sedate the calf before delivery.

Once the hind and calf have imprinted for about an hour, a check should be made. If bonding has started, the hind will have licked the calf dry and removed the membrane by this time and it may even try to stand and search for the udder. If the calf is still wet, and the hind shows no interest in it, it must be removed for artificial rearing. Colostrum may be collected as described later. If all is well, gates from the shed back to an adjacent paddock can be left open and the pair should again be left alone, for several hours if possible. Allowing a hind to move her calf in a passive manner is a better option than moving the calf, as the hind may refuse to accept it once she is out in the open and has not seen it lie down.

Elective cesarean section is an alternative delivery for red deer hinds used as surrogate mothers for purebred wapiti calves implanted by embryo transfer. The ethical issues surrounding this practice should be considered. It is also an option when vaginal delivery is not possible for other reasons. In most situations, this operation will be carried out under general anesthesia, although the use of epidural xylazine has been described.[1] In most cases resulting calves will be artificially reared, and the caveat on the dangers of rearing male cervid calves must be issued.

In the event that a dead calf is delivered, or if artificial rearing is necessary, colostrum may be collected from the hind. If a hind is recumbent after a cesarean section or mildly sedated while standing in a chute, she can be treated with oxytocin. A short while later colostrum can readily be collected with a 60-ml syringe with the hub end cut off and the plunger reinserted from the cut end, not the flange end. The flange end is then placed over the teat after greasing the udder to allow a seal, and gentle suction applied. Alternatively, a human breast pump may be used. As much as 750 ml has been collected at one time.[1]

WEANING

Choice of early weaning (pre-rut), late weaning (post-rut), or no weaning will depend on the individual farm and policies. All options have advantages and disadvantages, which relate to desired reproductive outcomes the following season, feed management, animal health program implementation, labor, etc. Thus, the decision is based on the aims and objectives of the individual farm. One reason for weaning is the age-specific difference in winter feed requirements between calves and adults, but pre-rut weaning is claimed to tighten the synchrony of breeding and calving. Weaning may be done when the calves are about 3 months old, in the first week or 10 days of September in the Northern Hemisphere. By this time, they will have been supplied with a creep feed arrangement and will be able to manage for themselves.[1]

Effects of Weaning on Reproduction

The advantages to a breeding program that accrue from early pre-rut weaning of red deer are that the hinds will have about a 3- to 5-week period before the onset of the rut. This will give them the opportunity to recover from the stress of the weaning itself, which lasts a few days, and from the nutritional burden imposed by the demands of lactation. They will be able to start putting on some weight and will be in the best possible nutritional condition at the start of the rut. This topic is covered more fully in the preceding section on body condition scoring. It has been reported that hinds weaned after the rut, and therefore lactating during the rut, were 0.5 of a BCS lower than pre-rut weaned hinds.[12] This, along with the possible effects of lactational hormones on ovulation, resulted in a delay in conception date of 12 days, but did not alter conception rate per se, although if early removal of the stag is desired, pregnancy rate may fall. Those authors noted that body weight and growth rate of post-rut weaned progeny were higher than those weaned pre-rut. However, in absolute terms, in the following season it is likely that the weights of progeny of pre-rut weaned hinds the year before will be equivalent because they would have been born earlier, and therefore be heavier at a given date as a function of birth weight. It is likely that feed quantity and quality will also influence the magnitude of differences between early and late weaning on a seasonal basis. Early weaning also increases breeding synchrony among hinds. This will bring forward the mean calving date, and in turn give the calf of the following year more time to grow to optimal weight before weaning. This reinforces the view that management decisions about reproduction should consider all implications and consider effects over two breeding seasons.

The longer gestation period of the wapiti relative to the red deer (247 ± 5 days versus 233 ± 7 days, respectively, in well-nourished hinds), and earlier rut onset, means that weaning at or about the beginning of September only provides the hind about 10 days to 2 weeks for recovery before the start of the rut. However, pre-rut weaning was associated with an advance in caving dates of 5 days as well as other benefits to the farming program, which included improved calving (3.3%), better weaning rates (2.6% in adult hinds and 13.4% in young hinds), decreased calf mortality rates (3.2%), and improved growth to 200 days (8%).[13]

Weaning Practices

Weaning can be pre-rut, post-rut, or natural (no management-imposed separation). A survey of wapiti farming practice in western Canada shows that both pre- and post-rut weaning is carried out, and that some farmers do not wean at all. It is common practice on intensively managed red deer farms to wean calves before the rut, and this is considered essential if hinds are to be entered into an artificial breeding program. Hinds in poor body condition, due to undernutrition, or that have been permitting multiple suckling should definitely have progeny weaned if they are to conceive in the early part of the breeding season.[9,12]

Three techniques for pre-rut weaning have been investigated. In remote weaning, which has been the traditional practice among beef farmers, and was adopted by wapiti and red deer farmers, calves are abruptly weaned at about 100 days of age and removed to a location remote from their dams. Interval weaning involves the shifting of a few hinds each day over a 10-day period to an adjacent pasture, and then to a remote pasture. Contact weaning involves weaning of the calves into a paddock with which they have been previously familiarized, adjacent to the paddock in which their dams are held, separated only by a reliably calf-proof fence. In two separate studies, interval and contact weaning were reported to be more welfare-friendly than remote weaning, with calves demonstrating fewer signs of distress. However, no differences in calf performance between trial groups were reported.[30,31] When comparisons were made between remote weaning, and calves weaned into unfamiliar paddocks, separated by 100 m from their dams, the latter showed more signs of distress than those moved a distance of 2 km.[32]

Weaning Rates

Weaning rates, for practical purposes, may be described as calves weaned/hinds joined to the stag, calves weaned/hinds pregnant at scanning early in gestation, or calves weaned/calves born. Each ratio gives different information. It is important to standardize terminology when comparing rates between farms, age groups, and species, because the effects of gestational and perinatal losses may be significant on many farms.

Data for New Zealand red deer farm weaning rates (calves weaned/hinds scanned pregnant) from 81 herds reported in several large surveys show primiparous hinds weaned at an average of 86.2% ranging from 64% to 100% between herds.[11,28] By comparison, adult hinds weaned at an average of 91.5% (range 78% to 100% between herds). When the effect of pregnancy rate is included, overall reproductive efficiency (calves weaned/hinds joined to stag) averaged 63% to 77% and 81% to 89%, respectively, for primiparous and adult hinds over 2 years.[28] Similar data from another study show ranges of 68% to 75% for primiparous hinds, and 80% to 88% for adult hinds, respectively.[11] Thus, there is enormous variation in reproductive efficiency among farms, ranging from 25% to 92% and 69% to 94%, for primiparous and adult hinds, respectively, indicating that management plays a pivotal role in reproductive outcomes.[27] Loss rates during gestation ranged from 0% to 2.5% (average 1%), indicating that the majority of losses between conception and weaning are periparturient.[11,28]

Data on weaning rates have not been as extensively studied in North America. In an on-farm survey of performance of farmed wapiti among 50 producers in Alberta involving 1084 hinds, weaning rates of 73% for 2-year-old and 91% for adult hinds resulted in an 88% weaning rate overall.[13]

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CHAPTER 129

Reproductive Management of Fallow Deer

ROBERT C. MULLEY

Fallow deer are the most widely distributed deer in the world and are primarily farmed for meat. Venison from fallow deer is fine grained and cut sizes are particularly suited to the restaurant trade, which usually prefers chilled rather than frozen product. To provide year-round availability of chilled product of consistent quality, a range of reproductive management strategies and production techniques, including selective slaughter of males and females at different times of the year, castration of some males for slaughter during the breeding season, and the use of hybridization, is required.

Genetic selection for reproductive success and growth and development to suit market criteria will further contribute to economic success provided that animal health and husbandry benchmarks for a particular region are adhered to.

Velvet antler is regarded as a by-product of fallow deer farming, but the amount of velvet antler produced by an adult buck each year rarely exceeds 500 g, and the financial return to the farmer after the costs of removal from the animal and sale levees make it a low return product. However, for management reasons (e.g., injury to breeding females, animal handlers in yards, etc.) the antler should be removed from fallow bucks anyway, and if this procedure coincides with antler growth at a highly saleable grade, then the price differential between costs and returns may favor the farmer. The antler cycle is strongly linked with reproductive cycles, and effective management of this species for production of venison necessarily involves consideration of the antler cycle in reproductive management strategies.

A number of factors need to be considered for the development of an efficient fallow deer farm. Assessment

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A number of factors need to be considered for the development of an efficient fallow deer farm. Assessment

of body score of both breeding and slaughter stock combined with information on nutritional requirements of different age and sex classes of stock at various stages of the production cycle are essential elements of reproductive management on both stud and commercial farms. The integration of information on reproductive biology, behavior, nutrition, genetics, lactation, and animal management of farmed fallow deer provided in this chapter will assist practitioners to advise owners of both small and large farms on how to optimize production for maximum profit.

THE BREEDING SEASON

Fallow deer exhibit highly seasonal patterns of reproduction calibrated by photoperiod. They are short-day breeders, with the rut (peak of mating activity) occurring in October/November in the Northern Hemisphere (NH) and April/May in the Southern Hemisphere (SH). Breeding pattern within regions is highly synchronized in any one year, and reproductive management of this species on pastoral farms must therefore align feed supply with seasonal demands of late pregnancy and lactation. Patterns of reproduction also vary slightly at different latitudes, and local benchmarks for reproductive behavior and onset of the rut need to be established. Description of the estrous cycle, estrus, length of the breeding season, and gestation length are provided by Asher in Chapter 123.

REPRODUCTIVE BEHAVIOR

Bucks

The breeding season is enormously stressful for fallow bucks. Potential sire bucks should be clinically examined by a veterinarian well before the start of the rut for detection of abnormalities, because once the rut commences they become extremely aggressive toward other deer if confined in handling yards and injuries can occur. Bucks will fight vigorously during the pre-rut period to establish dominance, and in very large paddocks and in the wild will sometimes establish stands, which they mark by thrashing small trees and digging rutting scrapes. In confinement bucks will walk the boundary of their fence line incessantly, especially in small paddocks (1–3 hectares) and can cause deep erosions from this activity in wet conditions. In small enclosures bucks give the impression that they are herding does because there is no real opportunity for lekking to occur. Even when the herd of does is resting the dominant buck will be moving continually to defend the harem against competitor bucks or to check does for estrus.

If bucks are placed in adjoining paddocks they will fight vigorously through a fence and can make large holes in it. The fighting will also create holes in the top soil on either side of the fence, as the bucks attempt to hold ground during their pushing and shoving. This enormous energy expenditure leads to rapid weight loss, and adult bucks can lose 20 to 30 kg in a period of 6 to 8 weeks just prior to and during the rut. This equates to a change in body condition score from 3.5 to 1.5 (on a scale of 5) and leaves the buck vulnerable to winter death syndrome if low body condition is combined with poor quality feed and cold-snap weather conditions.[7] Losses among sire bucks can be minimized by providing high-quality feed to sires immediately following the rut. This ensures protection of valuable genetics in preparation for subsequent breeding seasons.

Bucks are capable of breeding past the age of 15 years, but in the wild and on farms in competition with other bucks, they are unlikely to hold a harem beyond 7 to 8 years of age. Rapid turnover of breeding bucks on farms is desirable to reduce the intergeneration interval and maximize genetic gain through selection.

Does

Fallow does reach puberty weight prior to 16 months of age, but usually do not conceive until that age because of the acutely seasonal photoperiod effect that induces the onset of estrus. Occasionally precocious females will reach puberty weight by 8 months of age and conceive, but this should be avoided by careful management because of the impact of pregnancy on growth of the doe and the long-term impact on lifetime reproductive performance.

Estrous behavior in does appears quite passive compared with that of other livestock species.[1] It is rare for does in estrus to mount other does or be mounted, and the most obvious characteristic of fallow deer estrus is simply that a doe will stand to be mounted by the buck. A nonestrous doe will generally avoid close contact with the rutting buck and will run short distances if a buck approaches.

The onset of estrus can be very abrupt, although self-grooming, more activity than normal in the form of walking and fence-pacing, and reduced time spent feeding are indicative signs. Does can cycle for 3 to 6 months if not mated, at intervals of 21 to 25 days from early to later in the breeding season, respectively. Interest shown by both bucks and does in mating wanes remarkably after about the fourth estrous cycle of the breeding season, and it is uncertain whether this is due to weakening pheromonal signals associated with estrus or some other reason.

REPRODUCTIVE BIOLOGY

Fallow deer have a highly synchronized mating season. The does are polyestrous but most (~95%) conceive in their first or second estrous cycle. Pregnancy testing using ultrasonography is 100% accurate from 50 days of gestation, and a high level of accuracy is achievable earlier than this by experienced operators. Rapid fetal growth takes place in the third trimester of pregnancy when daily energy requirements increase by 25%. Basic information on the reproductive biology of fallow deer is presented in Table 129-1.

The buck-doe ratio used by most fallow deer farmers is 1:40 for experienced adult bucks and 1:20 for rising 2- and 3-year-old bucks. Higher buck-doe ratios can lead to subfertility in males and prolong the breeding season. This may lead to the birth of late fawns (conceived in

Table **129-1**

Fallow Deer Reproduction

Feature	Description
Onset of puberty	Does: 16 months (weight dependent, 35 to 40 kg) Bucks: 14 months (motile sperm first detected)
Onset of breeding season	Mid-April Southern Hemisphere, mid-October Northern Hemisphere
Duration of breeding season	6 estrous cycles in adult does 4–5 estrous cycles in younger does
Duration of rut	6 weeks (2–3 estrous cycles)
Length of estrous cycle	21 ± 0.6 days (1st cycle of breeding season) 25 ± 1.5 days (last cycle of breeding season)
Length of estrous	12 to 24 hours
Matings per estrous	Repeated mounting (16 ± 7) prior to final ejaculatory mount
Courtship	4 to 50 minutes (mean ± SD = 15 ± 10)
Gestation length	234 ± 2 days
Onset of fawning	Last week of spring
Duration of parturition	30–180 minutes from appearance of membranes
Fawning rate	88–92% of does joined
Male-female ratio at birth	1 : 1
Weaning rate	80–84% of does joined
Birth weight	Females: 4–4.2 kg Males: 4.2–4.6 kg
Twinning rate	<1 : 200

fourth or fifth estrous cycle), and these fawns will grow slower than their counterparts, be subject to winter conditions earlier in their development, and usually do not attain the adult body weights expected. Late fawns might not conceive at 16 months of age as expected, because their body weight will not be sufficiently high by then to support the onset of puberty, pregnancy, and then lactation. Fallow does are highly fertile and will continue to breed after 12 years of age, depending on conditions, although there is some evidence that they are most efficient between 3 and 8 years of age.[10]

Fallow does will usually miss one or more breeding seasons throughout their reproductive life even when environmental conditions seem favorable.[10] Stress may play a role in inducing this reproductive spell, and may originate from the heavy demands of lactation in drought years or from feeding the rapidly growing male fawn through to weaning the previous year, from introducing deer to new surroundings at the start of the rut, or changed environmental conditions.

Fawn growth rates between 100 and 200 g/head/day are usually attained in the first 12 weeks of life, but slow to average between 50 and 100 g/head/day for the following 9 months up to yearling age. Growth profiles for female and male fawns up to 12 months of age are shown in the annual management calendar in Table 129-2. Castration of males at 6 months of age reduces growth rate by 10% compared with their male counterparts, but this is compensated for by increasing ease of management of slaughter animals during the following breeding season.

Accurate records of reproductive performance and growth rates of progeny are essential for selection of high-producing animals and for sire comparisons in single-sire mating systems. Breeding does should be replaced at the rate of 20% per annum, and the intergeneration interval for breeding bucks should be as low as is feasible to maximize the genetic gains resulting from genetic selection.

WEANING

Weaning is a management procedure that separates breeding females from their progeny, and is usually carried out to (i) simplify management of doe mating groups over the rut, (ii) provide preferential feeding for juvenile animals during the winter, and (iii) allow easy access to young animals for animal health and welfare management once they are nutritionally independent of their dam, without disturbance to breeding herds.

Weaning can be carried out pre-rut or later in the breeding season in fallow deer, as lactation does not appear to inhibit ovulation and conception. However, the procedure should not be carried out without consideration of factors/stressors that can impact on behavioral and growth changes in weaned animals and their dams, such as unfavorable weather patterns (drought, wet, cold), body condition of dam or fawn, and introduction to unfamiliar surroundings of fawns, in particular after separation from their mother. If does become emaciated due to poor nutrition during lactation, it is probable that subsequent reproductive performance will be low if poor body condition is maintained throughout the rutting period, and pre-rut weaning is recommended under such conditions.[1] Studies have shown positive effects of pre-rut weaning on hind conception date and hind condition in red deer in New Zealand, but it was acknowledged that weaned calves grew more slowly.[14]

Weight loss occurs in fawns immediately after weaning and persists for 2 to 3 weeks whether the procedure occurs pre-rut or post-rut. Comparative evaluation of weight recovery in fallow deer weaned pre-rut compared with deer weaned post-rut has shown no difference between the groups by 12 to 14 months of age owing to compensatory weight gains that occurred during spring (9–12 months of age).[11] However, the availability of nutritious feed during this time is necessary to facilitate weight recovery. Minimization of weight loss at weaning is assisted by (i) weaning dams from their fawns so that fawns remain in familiar surroundings, (ii) adding two or three older animals of quiet temperament and who are used to human contact to the group of weaned deer, to assist in training of weaned deer to use of laneways, gates, and concentrate feeding points if required, and (iii) moving dams out of calling range of their fawns for 3 to 4 weeks. This may not be possible if deer are being weaned indoors, where appropriate strategies may need to be developed according to the size of the facility.

Controlled mating practices may require considerable pre-rut sorting and handling of breeding stock, and under

Table **129-2**

Annual Management Calendar

Month	Adult Bucks	Adult Does	Rising 2-Year-Olds	Fawns
1st month of summer: December (SH) June (NH)	Velvet bucks at "A" grade stage of growth Remove velvet from 2-year-old stud bucks	Monitor fawning and ensure adequate high-quality feed Lactation requires 20–24 MJME/head/day Record birth weights for stud records	Remove velvet from 2-year-old bucks Last year's fawns now regarded as rising 2-year-old animals	Sell animals reaching BCS and market weight requirements Weigh animals for selection data New fawns now being born
2nd month of summer: January (SH) July (NH)	Remove antler regrowth Veterinary examination of breeding bucks Record weight and BCS Vasectomize teaser bucks for use in AI program if needed.	Lactating does need 20–24 MJME/head/day. Provide feed supplement if required Catch, ear tag and weigh fawns born late	Remove antler spikes Record weight and BCS of deer to be sent for slaughter	Fawns start eating hard feed from 7 weeks of age
3rd month of summer: February (SH) August (NH)	Provide quality feed to bucks in preparation for the rut Bucks easily stressed late in month Yarding and handling procedures should be minimized	Lactating does need 24 MJME/head/day Provide feed supplements if required, to maintain BCS 2.5–3	Weigh and BCS animals for slaughter Provide high-quality feed (10 MJME/head/day) to does about to be mated for the first time	Fawns eating up to 5 MJME/head/day hard feed plus milk Growth rates of buck fawns 180–280 g/day Growth rates of doe fawns 120–200 g/day
1st month of autumn: March (SH) September (NH)	Select breeding bucks and introduce to does in mating groups Avoid placing bucks in adjoining paddocks with shared fence line as they will fight incessantly	Pre-rut wean Vaccinate does Record lactation status (wet and dry) of does Cull dry and poor temperament does Build doe BCS to 3 prior to joining Regular handling of does for AI to habituate to yards and quieten	Weigh and BCS does to be joined Minimum weight for European does is 38 kg, and hybrid does 40 kg Sell surplus does and castrated bucks for venison	Wean fawns if this procedure is carried out pre-rut Wean all fawns over 16 kg, vaccinate with polyvalent clostridial vaccine Weigh, sex, and tag animals if not done at birth Provide high-quality feed. Fawns now eating 10 MJME/head/day
2nd month of autumn: April (SH) October (NH)	Mating commences Provide high-quality feed to standby bucks and breeding bucks even though their VFI is greatly reduced Put teaser buck with does being prepared for AI	Feed high-quality feed to maintain BCS 3 at joining Natural mating commences Insert progesterone devices for synchronization of estrus in does for use in AI program Does will lose weight for 2–3 weeks after weaning	Minimum weight of 38 kg and BCS of 2.5 for does to be joined Sell castrated bucks and surplus does for venison	Best feed available for weaned fawns 10 MJME/head/day and 15% protein for best growth Will lose weight for 2–3 weeks after weaning 2nd vaccination with polyvalent clostridial vaccine Anthelmintic drench may be required in wet years
3rd month of autumn: May (SH) November (NH)	Replace breeding bucks with replacement buck for third estrous cycle Keep teaser buck with does that have had AI for 2 weeks after procedure, then replace with entire buck	Maintain does at BCS 3 Remove devices for synchronizing estrus and AI does Sell cull does for venison	Sell surplus does and castrated bucks for venison	High-quality feed to maintain growth rates of 80–100 g/day (bucks) and 65–85 g/day (does) Anthelmintic drench in wet years if required

Table **129-2**

Annual Management Calendar—cont'd

Month	Adult Bucks	Adult Does	Rising 2-Year-Olds	Fawns
1st month of winter: June (SH) December (NH)	Remove all breeding bucks from mating groups in first week of month High-quality feed for rest of winter to ensure survival and optimum velvet production	Doe mating groups can be combined for ease of management Monitor BCS closely and feed to maintain at score 3	Sell surplus does and castrated bucks for venison	Castrate bucks for slaughter in following winter Weigh and calculate growth rate since weaning Animal health check Feed supplement if required
2nd month of winter: July (SH) January (NH)	Provide quality feed to ensure optimum velvet production and recover BCS post-rut Require 20 MJME/head/day	Pregnancy test does and cull dry does Feed >10 MJME/head/day to all pregnant does Supplement if required	Feed 10 MJME/head/day to all pregnant does Supplement if required	Best available feed to maximize growth Weigh and record growth for selection
3rd month of winter: August (SH) February (NH)	Feed to appetite with high-quality feed Protect from weather extremes Record dates of antler casting	Regular check of BCS of does, any decline to be addressed with high-quality supplement Pregnancy test does that were not detectably pregnant last month	As for adult does All surplus castrates to be sold	Weigh and record growth Health check Record spike initiation on bucks
1st month of spring: September (SH) March (NH)	Record antler casting dates Provide high-quality feed	Sell cull does for venison Monitor BCS and maintain at 3 with supplements if necessary	Sell culls for venison Pregnant does treated same as adult does	Weigh and record growth and development of antler spikes
2nd month of spring: October (SH) April (NH)	Separate bucks into velvet mobs on basis of antler casting dates Give high-quality feed for optimum velvet production	Vaccinate with polyvalent clostridial vaccine Separate into fawning mobs Keep AI groups separate for ease of fawn identification Feed requirement now 13 MJME/head/day	As for adult does	Remove early antler spikes
3rd month of spring: November (SH) May (NH)	Velvet bucks at "A" grade stage of velvet growth Maintain on high-quality feed Sell cull and surplus breeding bucks	Maintain feed at >13 MJME/head/day Place does in fawning paddock early in month Ensure adequate shelter and water First fawns born in last week of month Record birth weights for stud records	As for adult does	Sell precocious bucks and hybrids for venison Remove antler spikes Weigh for selection data

AI, artificial insemination; BCS, body condition score; MJME, Megajoules of metabolizable energy; NH, Northern Hemisphere; SH, Southern Hemisphere.

these circumstances fawns are better removed from the breeding herd. The opportunity to preferentially feed weaned deer, and to carry out routine animal health management, without disturbance to breeding animals is facilitated by pre-rut weaning. In situations where concentrate feeding of deer is routinely practiced such as in-winter systems and indoors it is likely that young animals will not compete equally for feed with adult animals and their growth and development will not be optimal unless they are managed separately to adult stock. However, under extensive pastoral conditions where access to feed is not a limiting factor, where the mating policy involves joining a number of breeding bucks with the total breeding herd, and where reproductive performance data are not routinely recorded, separation of sub-yearling animals can take place in late winter/early spring without apparent production consequences.

There is little evidence in fallow deer to support the notion that either pre-rut weaning or post-rut weaning leads to improved reproductive management, if feed is available and disease or husbandry and management do not create stress that compromises growth and development in fawns or reproductive performance in does. Seasonal conditions and production targets should therefore dictate when weaning takes place under pastoral conditions.

DYSTOCIA

Dystocia is not common in fallow deer, and is most likely to be seen in does fawning for the first time. Birth weight in fallow deer is usually 9% to 11% of doe mating weight but may be as high as 12% in some animals. This proportionate weight appears to be easily accommodated by the dam at parturition, and birth usually occurs between 30 and 120 minutes from the appearance of membranes. Disturbance of the herd will prolong parturition, and intervention should not be considered within 3 hours. Where intervention is required the doe should be quietly isolated from the rest of the mob if possible, as major disturbance can lead to complications with the rest of the does, including mismothering or interrupted parturition.

Does with dystocia may need to be tranquilized before assistance is given, and it is usual in such cases that little interest is shown in the fawn if born alive. The fawn can be artificially reared (see lactation), and goat colostrum is a good substitute if deer colostrum is unavailable from the doe. If the doe is strong enough after delivery of the fawn and shows interest in grooming it, she should be left alone in a quiet place for bonding to occur. Fallow does are good mothers and once bonding has occurred, the doe and fawn can be returned to the rest of the herd.

NUTRITION AND BODY CONDITION SCORE

The interrelationships between nutrition, body condition score (BCS), and reproductive performance are well established for ruminants, including deer. The highly seasonal reproductive patterns in temperate species of deer leads to predictable body weight fluctuations at certain times of each year in adult animals, particularly males, and lactation also corresponds with dry summer conditions in many areas of the world where deer are farmed. Farmers must therefore set minimum body condition scores at strategic points of growth and reproduction to maximize overall productivity. Setting target or minimum body condition scores provides constant feedback on nutritional adequacy and also provides farmers with an insight to future feed requirements. The use of strategic feeding in conjunction with routine evaluation of body condition score promotes efficient resource management and should lead to higher profitability resulting from more consistent reproductive performance and slaughter animals grown to specification.

Seasonal variations in energy intake, and the annual energy intake for European fallow deer (E) bucks, does, and haviers (castrated male deer) and $^1/_4$ bred hybrids ($^3/_4$ European and $^1/_4$ Persian) (H) between 10 and 21 months of age are shown in Table 129-3, and daily energy intake and live weight change for the same animals are shown in Figure 129-1. The pronounced reduction in voluntary feed intake and associated reduction in live weight in bucks during autumn corresponds with the rut (peak

Table **129-3**

Seasonal Variation in Energy Intake (MJME/day) for Fallow Deer*

Sex of Animal		Mean Live Weight at 10 Months Old (kg)	Spring	Summer	Autumn	Autumn	Annual Intake (365 days)	Mean Live Weight at 10 Months Old (kg)
				MJME/day			MJME/head	
Bucks	E	33.0	14.5	14.3	12.1	13.4	4986	51.8
	H	38.5	14.9	15.8	13.2	15.1	5431	60.8
Does	E	26.5	8.8	9.3	10.1	7.1	3201	32.2
	H	28.3	9.6	11.6	11.2	8.2	3710	35.2
Haviers	E	28.6	12.3	10.9	10.3	10.8	4047	47.0
	H	29.8	12.8	12.7	11.1	11.8	4446	48.8

*For deer of different sexes from 10 to 21 months of age. Data for European (E) and hybrid (H) ($^3/_4$ European, $^1/_4$ Persian) genotypes are represented.
MJME, Megajoules of metabolizable energy.

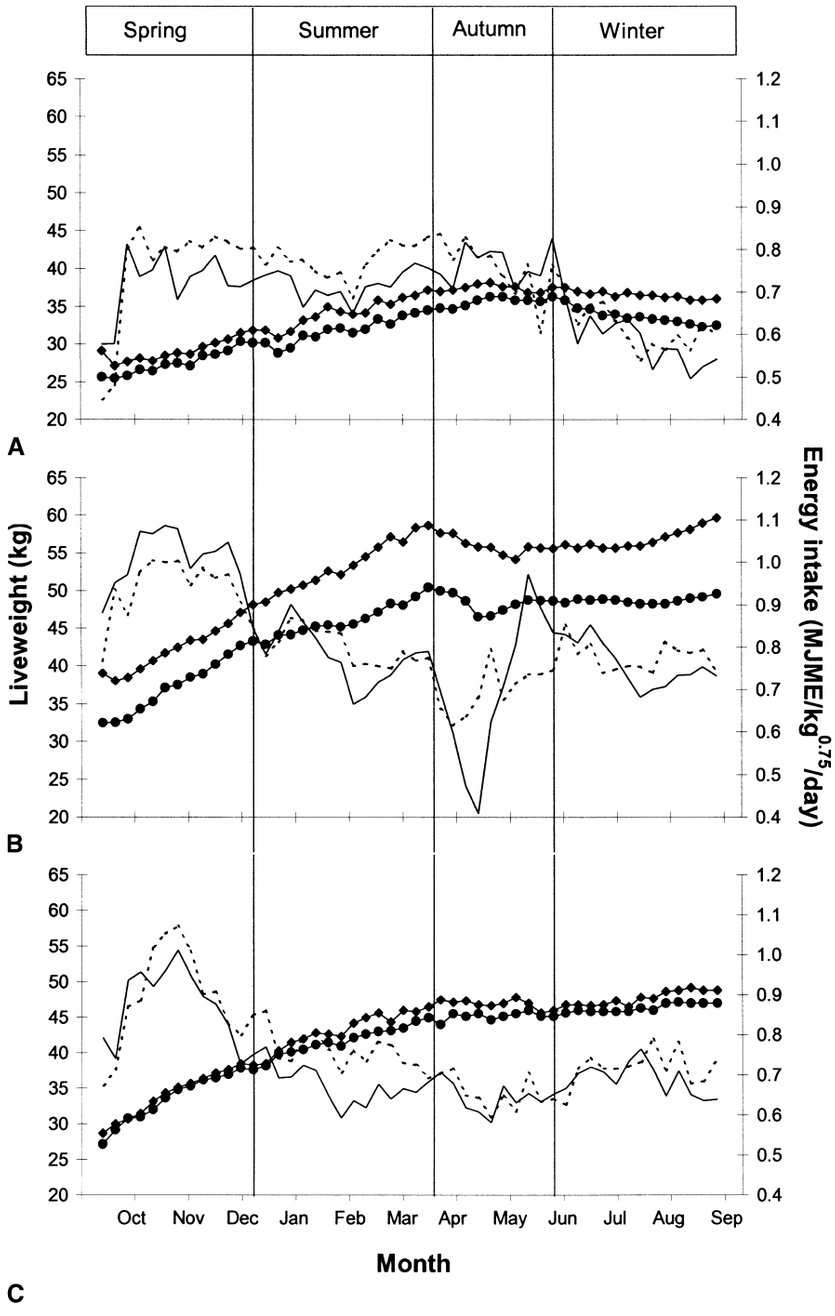

Fig. 129-1 Mean live weights and energy intakes of individually penned (A) does, (B) bucks, (C) haviers of the European (E) and $^1/_4$ bred Persian hybrid (H) genotypes of fallow deer over four seasons: live weight (kg). ●E; ◆H: Energy intake (MJME/kg body weight$^{0.75}$/day).

breeding period), and this reduction is significantly increased in older males, particularly those being used for breeding when up to 30% of live weight may be lost during this time. Calculated energy requirements for adult fallow bucks of different ages and weights over seasons of the year are shown in Table 129-4, although these data are interpolated from information derived for red deer stags in the winter in New Zealand.[3]

The metabolizable energy (ME) requirements for European and hybrid fallow does between 45 and 55 kg live weight in the last two trimesters of pregnancy and the first 12 weeks of lactation are shown in Figure 129-2. Accelerated energy intake in the third trimester corresponded with rapid fetal growth, and the total ME intake during the first 12 weeks of lactation is more than double the requirement for adult does in midpregnancy (winter). Feed supply in late pregnancy and lactation is critical for maintenance of optimal reproductive performance, and recent work with red deer showed that restricted daily energy intake during late pregnancy was associated with loss of body condition score and increased gestation length.[7]

Table **129-4**

Calculated Energy Requirements (MJME/day) for Breeding Bucks*

Age of Sire Bucks (Years)	March Live Weight (kg)	Autumn 100 days	Winter 65 days	Spring 100 days	Summer 100 days
Rising 3	65	17.0	16.1	18.1	18.1
Rising 4	85	20.7	19.6	20.3	20.3
Rising 5	105	24.3	23.0	24.1	24.1

*For bucks of different ages over seasons of the year.
From Asher GW: Growth and feeding management of fallow deer in New Zealand. Proceedings of the First World Forum on fallow deer farming, 1993, pp 67–72.
MJME, Megajoules of metabolizable energy.

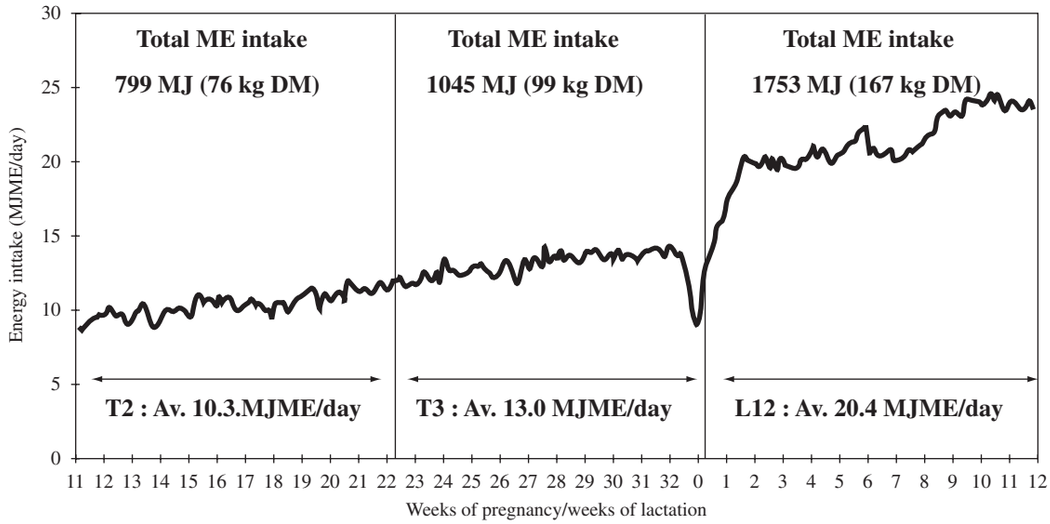

Fig. 129-2 Metabolizable energy intake of fallow does over the second (T2) and third (T3) trimesters of pregnancy and first 12 weeks of lactation (L12). (From Flesch JS: Nutritional requirements of pregnant and lactating fallow deer (*Dama dama*). Doctoral thesis, University of Western Sydney, Sydney, Australia, 2001.)

Low quality feed during late pregnancy and lactation in fallow deer has been shown to be correlated with the occurrence of fawns of low birth weight and lower than expected survival to weaning.[6,9] However, recent attempts in fallow deer and red deer to affect fetal growth by restricting feed intake to does in mid- to late pregnancy were unsuccessful unless body condition scores were lowered significantly, because of strong intrinsic mechanisms to compensate for environmental extremes. Condition score monitoring needs to be done on a regular basis to be effective, as the manifestation of poor nutrition may not be detected until it is too late to address.

Weaned fallow deer commence eating hard feed from 6 weeks of age, and by 16 weeks will consume 10 megajoules of metabolizable energy (MJME)/head/day, or 0.95 MJME/kg$^{0.75}$/day of feed comprising 14 MJME/kg dry matter and 15% protein if available to them. The feed intake associated with rapid growth in this age group equates to 3.4% of body weight for 25-kg animals. Hence, it is possible that on less palatable and low digestibility feeds, "gut fill" may prohibit fawns from ingesting sufficient feed to extract the required ME and crude protein for optimal growth.[7] The high daily feed requirement of weaned fallow deer from 16 weeks of age has seldom been accurately estimated by farmers in the past, and feed budgets for autumn and winter in particular, when rapidly growing weaned deer are being fed through their first winter, need to be carefully calculated.

Adult breeding bucks in low body condition following the rut require careful management and access to high-quality feed. Inappetence during the rut may reduce the live weight of a 100-kg buck pre-rut to 70 kg post-rut, and reduce the condition score from between 4 and 5 in late summer (5 point scale) to 2 or lower post-rut. Any reduction below a body condition score of 2 increases the vulnerability of breeding bucks to winter death syndrome, which refers to death resulting from a combination of poor quality feed, low body condition score, and cold-snap weather conditions. Valuable genetics in breeding bucks must be protected by availability to high-quality

feed and careful management post-rut to avoid such losses.

A body condition scoring system for fallow deer has recently been developed and this will greatly assist animal health professionals, wildlife biologists, and deer farmers to manage herds of fallow deer for production and reproductive performance.[7]

Body Condition Score

A 5-point body condition scoring system for a fallow deer has been developed and can be summarized as follows[7]:

GRADE 1: Very Poor Condition (Emaciated)
Animals in this category are emaciated through malnutrition, old age, or parasitism, disease, or injury. The wings of the pelvis are extremely prominent with no palpable fat over the rump, which is concave in appearance with little muscle coverage. The spine is highly palpable, giving the body an angular appearance. The ribs may also be palpable or visible through the skin. Does in this condition are usually infertile.

GRADE 2: Poor Condition (Lean)
Animals in this category are also obviously thin. Adult bucks are usually BCS 2 after the rut, and lactating does may be seen in BCS 2 in times of feed shortage. As with BCS 1, the wings of the pelvis are prominent and easily palpable. Rump areas are flat with slight tissue coverage. Sacral spinous processes are easily palpable, with the saddle having a slightly angular appearance.

GRADE 3: Moderate Condition
Animals in this category, are not undernourished as described in BCS 1 and 2, but still do not display prominent deposits of fat in certain areas of the body. BCS 3 should be a minimal score for breeding stock. The wings of the pelvis are not as prominent as BCS 1 and 2, but are still palpable with slight finger pressure. The spine is also palpable, but is slightly enveloped in tissue. The body has a more rounded appearance. The rump is still flat, although a greater mass of muscle tissue is felt with firm pressure.

GRADE 4: Good Condition
Animals in this category are considered to be in good condition. The wings of the pelvis are rounded, and can be palpated under a thin layer of fat. The spine is also enveloped in fat, and may be felt only with firm finger pressure. The body now has a rounded appearance over the saddle, with no clear delineation between the torso and pelvic area of the animal. The rump has considerable fat coverage, and is slightly convex. Brisket fat is now visible and easily palpated. Adult bucks usually reach and may exceed BCS 4 in late summer. Adult breeding does will reach BCS 4 in late spring or when not pregnant and lactating for at least 12 months.

GRADE 5 Very Good Condition (Fat)
Animals in this condition are considered too fat for commercial purposes. The metabolic debt of pregnancy and lactation usually prevents does from attaining this degree of condition, although bucks with abundant feed may reach BCS 5 over summer. Castrates, if not slaughtered by their second summer, may also reach this level of fatness. The wings of the pelvis are concealed in fat and cannot be palpated. Spinal processes are also enveloped in a layer of fat and not felt at palpation, giving the animal a very rounded appearance. The rump is extremely well covered and convex. Brisket fat is highly visible and easily palpated from the thorax to the distal end of the sternum.

GENETIC SELECTION

Genetic variation is the most important precondition for any selective breeding program, but it has been shown that there is very little genetic heterozygosity in fallow deer world wide.[13] Further, for recently domesticated species such as deer, heritability of production traits will depend on the genetic history of the species, both in terms of its previous abundance and the selective forces that it has experienced in the past. Although there is little genetic variation in fallow deer, some quantitative progeny test data collected in New Zealand show that there is enough genetic variation in the population for traits of economic value and for some traits the heritability (h^2 estimate) is similar to that found in other farm animals[4] (Table 129-5). Although pelage color is 100% heritable in fallow deer, there is no evidence that coat color variation is linked to production traits that a farmer might want.

Although complex economic traits are controlled by many genes it is clear that increased selection intensity (i.e., selection of very few sire bucks from a large pool) and reduced generation interval (i.e., rapid introduction of young bucks and does to breeding herd, and turnover of older animals) will lead to cumulative genetic improvement in fallow deer. The ongoing improvement of breeding value in a line of animals can be quantitatively defined if desired production traits are accurately measured and recorded. However, as for other animal production systems, genetic selection will only achieve results if the following occurs:

- The breeding objectives are clearly defined.
- Only two or three breeding objectives are tested at once.

Table **129-5**

Heritability Estimates for a Range of Traits in European Fallow Deer

Trait	h^2 Estimate	
Pelage color	1.0	High heritability
Growth traits	0.2–0.4	Inadequate heritability
Antler size	0.3–0.4	Moderate heritability
Carcass fatness	0.3–0.4	Moderate heritability
Fawn survival	0.1–0.2	Low heritability

From Asher GW, Langridge M (eds): *Progressive fallow deer farming*, 2nd ed. Hamilton, NZ: Ruakura Agricultural Centre, 1992, pp 79–94.
h^2, The efficiency of transmission of parental phenotypic superiority to the next generation.

- The traits are easily and accurately measured.
- The traits are inherited.
- The selection pressure on breeding stock is high.

Environment (E) and in particular nutrition play a major role in whether valuable genes (G) are expressed optimally. Breeding value is best determined through use of progeny testing where possible when purchasing breeding stock; otherwise, it is possible to pay large amounts for E when the cumulative and permanent G is sought.

Rapid genetic improvement for some commercially desirable traits may be more easily achieved through crossbreeding, and hybridization between the European fallow deer (*Dama dama dama*) and the larger Persian fallow deer (*Dama dama mesopotamica*) is now commonly practiced in commercial fallow deer farms for production of animals for meat, and for production of breeding does with larger body size that can produce fawns with higher birth weights.

Reproductive success of farmed deer is often related to quiet temperament, and the quietest deer are also often the heaviest. Therefore, selection on temperament may produce larger and quieter deer, and if temperament is partly genetic, then the manifestation of this selection pressure is permanent.

HYBRIDIZATION

There are only two subspecies within the genus *Dama*, European fallow deer (*Dama dama dama*) and Persian fallow deer (*Dama dama mesopotamica*). Persian fallow deer readily hybridize with European fallow deer, and the mature body size of Persian fallow deer (adult males 145–150 kg; adult females 70–75 kg) is significantly larger than their European counterparts. Hybridization capitalizes on additive genetic effects from combining two genotypes, as well as the potential effects of heterosis.

Four additive breed effects result from hybridization of these two subspecies:

- Faster growth rates
- Larger mature body weight
- Increased weight at puberty
- Slaughter weight reached sooner

The hybrid progeny also show no difference in body composition or dressing percentage and hybrid breeding does are slightly more efficient in terms of feed utilization than their European counterparts.[12] Furthermore, concerns about the occurrence of dystocia resulting from mating large Persian bucks over the smaller European does were ill-founded, although this practice should take place only with mature adult does and not rising 2-year-old does.

The term hybridization should not be used when referring to crosses between European fallow deer sourced from different geographic locations world wide, such as England, Denmark, Hungary, Australia, New Zealand, etc. Although there may be some merit in accessing animals of exceptional quality from these various locations for use in breeding herds, they are merely geographic variants of European fallow deer. As stated earlier, there is only one hybridization opportunity within the genus *Dama*, and that is between the European and Persian subspecies.

FETAL AGING

Pregnancy diagnosis using ultrasonography is a routine procedure in fallow deer, especially in controlled breeding programs or for culling of dry does. Fetal and placental measurements can assist practitioners in determining gestational age, which can be used to inform management practices. Detection of pregnancy using ultrasound is possible in fallow deer after 30 days in the hands of experienced operators, but for most commercial purposes results obtained at 50 days or greater are unequivocal. The data in Table 129-6 provide some fetal and placental measurements for fallow does scanned at 50 and 65 days of gestation. After 65 days the fetus has usually descended into the abdomen of the doe, and measurement by ultrasound is no longer possible. However, necropsy of does at known gestational ages (Table 129-7) provides insight into fetal and placental growth that takes place later in pregnancy, and this information may be useful for veterinarians and wildlife managers investigating outbreaks of disease.

There is evidence that nutritional stress (30% reduction of voluntary feed intake per day) in fallow does in the last trimester of pregnancy affects fetal growth and significantly affects doe body condition score. This leads to the occurrence of fawns of low birth weight that are too weak to stand and suckle, and that die within 24 to 48 hours after birth. Greater than 50% of fawns less than 3.5 kg at birth die within 48 hours after parturition. There is no evidence that poor nutrition in late pregnancy prolongs gestation length in fallow deer, as has been shown in red deer.[2]

FAWNING

The fawning season of fallow deer is generally condensed into the last 2 weeks of spring and the first 4 weeks of

Table **129-6**

Fetal and Placental Ultrasonographic Measurements

	DAYS OF GESTATION	
Parameter	**50 days (*n* = 16)**	**65 days (*n* = 18)**
Placenta width (mm)	14 ± 2	27 ± 2
Crown rump length (mm)	32 ± 2	118
Fetal chest depth (mm)	12 ± 2*	—
Fetal head length (mm)	17 ± 1	28 ± 4

From Flesch JS: Nutritional requirements of pregnant and lactating fallow deer (*Dama dama*). Doctoral thesis, University of Western Sydney, Sydney, Australia, 2001.

Table **129-7**

Necropsy Measurements of Fetal and Placental Parameters for Fallow Deer at Known Gestational Ages

	GESTATIONAL AGE (DAYS)			
	42	**84**	**140**	**217**
Fetal weight (g)	4–5	105	900–950	4300–4900
Crown-rump length (mm)	38–42	135	250–270	440–460
Placental mass (g)	3	150–170	325–350	620–810
Placentome number	4–6	7–9	8–11	8–11
Total conceptus mass (g)	220–230	1180–1220	2850–3100	—
Number of animals	24	18	24	24

From Flesch JS: Nutritional requirements of pregnant and lactating fallow deer (*Dama dama*). Doctoral thesis, University of Western Sydney, Sydney, Australia, 2001.

summer owing to the high fertility of this species and highly synchronized start to the breeding season. Studies have shown that 95% of fallow does conceive in either their first or second estrous cycle of the breeding season, thus leading to the concentrated fawning pattern referred to. Fawns can be born later than this if bucks remain with does throughout the autumn and winter months, when persistently cyclic does can conceive at their fourth or fifth estrus, or if precocious doe fawns left unweaned from their mothers reach puberty weight by 8 months of age. However, fawns born in late summer/early autumn as a result of these late conceptions have low survival rates, grow significantly more slowly than their cohort counterparts if they do survive, and complicate management. It is becoming common practice for managers to remove sire bucks from breeding herds in early winter, effectively giving does only two to three chances to conceive.

An understanding of the normal parturient and maternal behavior patterns of fallow does is necessary in order to detect abnormal situations requiring attending. Fallow does go off their feed 2 to 3 days before parturition, and become increasingly restless until parturition. They will often disassociate from the main herd, pace the fence line in smaller paddocks, and as birth becomes more imminent, frequently lick their vulval region.[1] Does in small paddocks can be disturbed by curious or passing deer and this can prolong parturition, but this is unlikely in larger paddocks with lower stocking rates. However, parturition to completion of grooming of the newborn fawn usually takes 60 to 180 minutes on average, and if parturition is taking longer than this, obstetric intervention may be required.

Fawns will suckle within the first hour after birth, and will then find a resting place, which is usually some distance away from the doe. This is normal behavior and fawns found on their own should not be considered to have been abandoned. Does will return several times during each day to suckle their fawn. Under intensive conditions this could occur as often as seven to eight times during daylight hours, and four to five times under more extensive pastoral conditions.

In some parts of the world where deer are farmed, fawning coincides with deteriorating pasture quality and this can lead to poor growth rates and severe decline in doe body condition. Poor feed quality prior to parturition can also reduce fetal growth in the last trimester of pregnancy, leading to production of fawns of low birth weight. The strong correlation between low birth weight and perinatal death in fallow deer is well established.[1,6] Other causes of perinatal death in fallow deer are presented in Table 129-8.[6]

Table **129-8**

Causes of Perinatal Deaths and Associated Birth Weights of Fallow Deer Fawns

Cause of Death (Class)	Number	%	Birth Weight (kg) ± SD
1A	16	11.1	1.99 ± 1.05
1B	6	4.2	3.80 ± 0.48
1C	7	4.9	3.43 ± 0.87
1D	4	2.8	1.38 ± 0.25
2	3	2.1	3.80 ± 0.14
3	11	7.6	3.31 ± 0.88
4	62	43.1	2.86 ± 0.84
5	7	4.9	4.20 ± 0.80
6	4	9.7	4.02 ± 0.62
7	2	1.4	2.90 ± 0.71
8	12	8.3	—
Total	**134**	**100.0**	**3.01 ± 1.07**

Key: 1A, anteparturient death; 1B, parturient death, birth trauma obvious; 1C, parturient death, no obvious birth trauma; 1D, premature; 2, birth trauma, lungs aerated; 3, misadventure; 4, exposure/starvation; 5, mismothering; 6, infections; 7, malformations; 8, undiagnosed.

As a general principle, does should be familiar with their fawning paddock and given at least 2 weeks to acclimatize prior to the commencement of fawning. The fawning paddock should contain sufficient ground cover for fawns to hide in, and to be protected from environmental extremes of heat or cold. Feed available to does in early lactation needs to be abundant and of high quality to accommodate the twofold increase in energy intake that occurs immediately after parturition. Proximity to

water is also a key point and lactating animals should not have to go too far for water. Does will stay in close proximity to where their fawn is hiding for at least the first 10 days after birth.

In some circumstances it may be necessary to hand-rear a fawn, and this is easily accomplished. However, it is necessary to warn against hand-rearing of buck fawns, as they do not have any fear of humans as adults (from 16 months of age) and can be particularly dangerous with or without antlers. Hand-reared does remain very quiet and can be used to show leadership in laneways and sheds when training weaner age deer.

The composition of milk from does has been reviewed by Whitehead.[15] Compared with the domestic cow, deer produce a milk rich in energy, fat, and protein, but similar in lactose concentration. Cervid milk contains 19% to 26% dry matter, 6% to 11% fat, 6% to 10% protein, 3% to 5% sugar, and 1% to 2% ash. When compared at peak lactation, cervid milks are all reasonably similar in gross composition and more concentrated than domestic bovid milks. If a newborn deer has not had colostrum, then goat colostrum is a good substitute and can be readily stored in the freezer for anticipated use. A simple but proven formula for hand-rearing fallow fawns comprises 100 g milk replacer per 100 ml of water, 1 egg yolk, 5 g neosulphetan or tribisson, 20 g dextrose, 5 ml cod liver oil, and 5 ml multivitamin solution. Feed several times per day via bottle or bowl, at 30° to 40° C. Additional water should be provided at all times to prevent dehydration, especially in very hot climates or if alternative and more concentrated mixtures are used.

VENISON PRODUCTION

The usual measure of efficiency in a meat production system is the kg/ha turnoff of meat per annum. A slight complication in the production of venison from deer is that much of the product is ready for slaughter at the same time each year because of the acutely seasonal breeding pattern. To overcome this clustering of product availability, a range of strategies can be employed to provide carcasses of consistent quality year round. These strategies include hybridization, selective slaughter of surplus female stock, and castration.

Hybridization between European fallow deer and Persian fallow deer produces offspring that reach slaughter weights up to 2 months sooner than purebred European fallow deer for both males and females. Hence, from 9 months of age precocious hybrid bucks ($^1/_4$, $^3/_8$, and $^1/_2$ bred) are available for slaughter and produce carcasses from 27 to 30 kg. European fallow bucks reach equivalent slaughter weights from 11 to 12 months of age and hybrid does from 12 to 14 months of age. If a proportion of hybrid and European bucks are castrated each year, they produce venison of excellent quality and can be easily handled through slaughter premises during the breeding season when entire bucks are difficult to handle and produce venison of variable quality. Use of breeding and management strategies such as these allows producer flexibility to provide fresh or chilled product of consistent quality to the market year round. Preslaughter assessment of body condition score should be combined with animal live weight to ensure that only the animals suited to market specifications are slaughtered.

ANTLER MANAGEMENT

Antlers are grown annually by fallow bucks and are normally removed from animals on intensive farms for management reasons. If harvested at the required stage of growth, the entire growing antler, known as velvet, can be sold. Bucks with full hard antler can inflict serious if not lethal damage to other deer and livestock managers, can do enormous damage to trees by continual rubbing and territory marking during the breeding season, and can easily get caught up in stock fencing, which occasionally will lead to serious injury or death of the animal. In breeding bucks this is a serious and costly loss of genetic capital.

If velvet antler is to be removed, either for commercial gain or simply for ease of management, it must be done humanely by trained operators, and in many countries this requires veterinary supervision. Harvesting necessarily involves analgesia and standardized techniques for removal and animal restraint. However, even with good facilities and experienced personnel antler is easily damaged as a result of yarding. In one study 27 of 227 (11.9%) fallow deer handled through the yards for velvet antler removal had damaged antlers as a result of yarding.[8] Of deer with damaged antlers, 4 of 27 (14.8%) had damaged both antlers. Significantly more antlers (22/220 or 10%) harvested from 2-year-old bucks were damaged, compared with older bucks, and this reflects their relative lack of habituation to the process of yarding and handling. Saleable "A" grade velvet, as standardized by the market, can be harvested in late spring/early summer from adult bucks 2 years old or older, about 45 ± 3 days following antler casting. In this study the weight of "A" grade velvet antler harvested from fallow bucks increased annually until a peak at 6 years of age.[8] The data indicate that the average age of fallow bucks in herds developed for velvet production should be 5 years to achieve a sustained maximum harvest and to allow for development of replacement animals before the rapid fall-off in velvet yield after 6 years. The low annual yield of "A" grade velvet antler from fallow bucks and variable marketability of this product strongly influence whether the species is useful commercially for velvet production and for this reason it is not usual to see herds of fallow bucks kept for this purpose. However, given that the antlers should be removed from adult breeding bucks for management and safety reasons, they should be removed at the most commercially saleable stage to recoup the costs of antler removal and maintenance feed costs. Velvet antler weight approximately doubled between 2 and 5 years of age (Table 129-9).

The yields shown in Table 129-9 are baseline data and more highly selected strains of deer are now producing greater annual yields than those shown. Mature bucks of Danish and Hungarian strains in particular are likely to yield more.

Bucks grow an antler spike in their first year of life and this must also be removed for safe handling of deer to

Table **129-9**

The Mean (± SD) Weight and Length of Velvet Antler Harvested From Fallow Bucks of Known Age

Age (Years)	No. of Bucks	Mean Antler Weight (g)	Mean Antler Length (cm)
2	110	246 ± 50	13.4 ± 2.6
3	35	282 ± 64	14.2 ± 2.1
4	25	365 ± 54	14.1 ± 1.9
5	32	445 ± 72	16.4 ± 1.0
6	15	486 ± 72	16.3 ± 0.5
7	5	365 ± 43	12.7 ± 0.8
8	5	333 ± 36	

slaughter. Effective methods of removal include use of rubber ligatures in early growth phases in spring, lopping using long-handled horticulture cutters or a surgical saw after application of local analgesic in spring, or lopping of the calcified spike in midsummer using cutters. Local laws must be consulted prior to implementation of antler management procedures.

ANIMAL HEALTH

Deer are only recently domesticated to intensive agriculture. Fallow deer have adapted well to domestication and are now well established in a wide range of environmental and pastoral conditions world wide. They have adapted just as well to intensive indoor conditions as they have to extensive pastoral conditions, although different animal health and stress management procedures need to be applied. In general, fallow deer are susceptible to most of the diseases of other domesticated ruminants but appear to be remarkably disease resistant at this point in their domestication. This innate disease resistance is more fragile as stocking rate increases, and disease from parasites and other infectious diseases are more likely to impact on management systems with high stock density. Diseases such as acute parasitism, pasteurellosis, necrobacillosis, leptospirosis, and clostridial diseases have been recorded more frequently in fallow deer herds managed under intensive conditions. Conversely, fallow deer appear not to be susceptible to malignant catarrhal fever, which is a major disease threat in many other deer species, and also appear less susceptible to yersiniosis and lungworm. Facial eczema and ryegrass staggers also cause significant problems in fallow deer in many parts of the world, and need to be carefully monitored.

Owing to their recent status as domesticated animals, fallow deer still retain some of their "wildness" and need to be handled in specifically built facilities by experienced and confident livestock handlers to reduce stress. Habituation to the process of moving through yards and laneways is particularly important if stress-related injuries and production losses are to be minimized. This is particularly important for animals being prepared for use in artificial breeding programs in which expensive techniques and valuable genetics are being used. A high success rate (>75% conceptions) can be achieved using artificial insemination in fallow deer providing the deer are calm and well habituated to their surroundings and handling procedures.[5] Younger animals are usually more prone to stress, and combining older, experienced animals with good temperament into mobs of younger deer for initial training can be extremely beneficial.

KEY POINTS

- Fallow deer exhibit highly seasonal patterns of reproduction controlled by photoperiod.
- The rut is an enormously stressful period for bucks, and careful management and feeding of deer during the breeding season is required.
- Approximately 95% of fallow does conceive at either their first or second estrous cycle of the breeding season, at buck-doe ratios of 1:40.
- There is little difference between pre-rut weaning and post-rut weaning on fawn growth and doe fertility.
- Does should weigh 38 to 40 kg prior to mating at 16 months of age
- Fawn survival is strongly linked to birth weight, and weights below 3.5 kg are unacceptably low. Fawns over 4 kg at birth have 95% chance of survival to weaning.
- Body condition score rather than birth weight is the best indicator of nutritional sufficiency and reproductive capacity in adult deer.
- Hybridization of European fallow deer and Persian fallow deer provides farmers with increased production options for efficient venison production.
- Artificial insemination techniques for fallow deer are very effective and proved under commercial conditions, and are now a commonly used tool in genetic improvement programs.
- Strategic feeding of fallow does in the third trimester of pregnancy and during lactation is recommended for maintenance of long-term reproductive performance.

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CHAPTER 130

Reproductive Management of White-Tailed Deer

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Among deer, the whitetail is unique. Its reproductive timing can vary by as much as 6 months or more within the same hemisphere, it has a higher fecundity rate than most species of deer, it can reach puberty in its first year of life, and it can alter its fertility to meet favorable and unfavorable environmental conditions.

As with other deer species, reproductive timing is regulated to a large extent by photoperiod, and it has been demonstrated that photoperiod sets the endogenous clock of the whitetail. However, research also has demonstrated that the whitetail's reproduction is governed by a genetic clock. Because the whitetail has a geographic distribution that extends across two hemispheres and has adapted to dramatically different environments, ranging from tropical to desert to temperate to boreal forest, it above all deer has perhaps the most complex reproductive adaptations.

FUNDAMENTALS AND BIOLOGY

Puberty

Puberty can be reached as early as 6 months of age. Studies have demonstrated that, depending on nutritional conditions, up to 80% of doe fawns can conceive during their first year of life. Such rates have been common in the Midwestern farm belt.[1] However, in the southeastern United States, this rate drops to an average of 40% or less.[2] At the extremes of the range, puberty is generally delayed to the yearling age class. Nutrition is important and onset of puberty for fawns is dependant on body size. For northern races of deer, fawns must reach 80 to 90 lb, and for smaller southern races, body size of 70 lb or more seem to be required for puberty to occur in the first year of life.[3] Both protein and energy are

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CHAPTER 130

Reproductive Management of White-Tailed Deer

HARRY A. JACOBSON

Among deer, the whitetail is unique. Its reproductive timing can vary by as much as 6 months or more within the same hemisphere, it has a higher fecundity rate than most species of deer, it can reach puberty in its first year of life, and it can alter its fertility to meet favorable and unfavorable environmental conditions.

As with other deer species, reproductive timing is regulated to a large extent by photoperiod, and it has been demonstrated that photoperiod sets the endogenous clock of the whitetail. However, research also has demonstrated that the whitetail's reproduction is governed by a genetic clock. Because the whitetail has a geographic distribution that extends across two hemispheres and has adapted to dramatically different environments, ranging from tropical to desert to temperate to boreal forest, it above all deer has perhaps the most complex reproductive adaptations.

FUNDAMENTALS AND BIOLOGY

Puberty

Puberty can be reached as early as 6 months of age. Studies have demonstrated that, depending on nutritional conditions, up to 80% of doe fawns can conceive during their first year of life. Such rates have been common in the Midwestern farm belt.[1] However, in the southeastern United States, this rate drops to an average of 40% or less.[2] At the extremes of the range, puberty is generally delayed to the yearling age class. Nutrition is important and onset of puberty for fawns is dependant on body size. For northern races of deer, fawns must reach 80 to 90 lb, and for smaller southern races, body size of 70 lb or more seem to be required for puberty to occur in the first year of life.[3] Both protein and energy are

important. Protein is obviously critical to reaching minimum body size. However, it has been demonstrated that female fawns on high-energy diets had significantly higher levels of progesterone than did fawns on low-energy diets, regardless of protein levels in the diet.[4]

At the extremes of the range, whitetails may be programmed to delay puberty. Michigan researchers demonstrated that even when supplement-fed, doe fawns in the Upper Peninsula of Michigan did not become pregnant, whereas 60% or more of doe fawns in the farmlands of the Lower Peninsula commonly were pregnant.[5]

Buck fawns can be expected to show similar rates of puberty to those of doe fawns. In buck fawns, puberty is indicated by the appearance of small calcified antler buttons in their first winter. Because of the relationship between testosterone production and hardened antlers, these calcified antlers indicate puberty has occurred and these animals are normally capable of breeding and producing offspring. However, with both buck and doe fawns, timing of puberty and ability to breed and conceive offspring are generally much later in the year than is the case with the onset of adult breeding. In general, yearling does normally have fawns at least a month or more later than do adult does.

Seasonal Changes in Sexual Characteristics

Because the whitetail is a seasonal breeder there are marked changes in both secondary reproductive characteristics and reproductive anatomy. In effect, the white-tailed deer and other deer undergo pubertal changes every year of their existence. These changes are readily evident in secondary sex characteristics of the male with the onset of increased body size, swollen neck, and development of large antlers. As with other deer species, the annual calcification of antlers and the rub-out of velvet signal readiness to breed.

Changes in the reproductive anatomy of males are characterized by annual increases and decreases in secondary sex glands and the testes and epididymis (Fig. 130-1). These changes occur earlier in 2-year-old or older bucks than they do in yearling bucks (Fig. 130-2).

Changes in female reproductive characteristics are less obvious than in males. Enlargement of the ovaries, vagina, and uterus do occur just prior to the rut. Changes are most pronounced in fawn and yearling does, but enlargement and increased elasticity are also noticed in the primary reproductive organs of adult females. The uterus of the white-tailed doe is bifurcate. Ovulation is said to occur from 12 to 14 hours after estrus.[6] Implantation occurs about 30 days after conception.[3] Following ovulation, normally from one to three corpora lutea are readily visible as ovarian swellings on one or both ovaries. Corpora albicantia of pregnancy are readily visible as large pigmented scars in formalin-fixed and sectioned ovaries of does for at least 3 months following parturition. Corpora albicantia of pregnancy from previous years are often visible as smaller pigmented scars in fixed tissue. Corpora albicantia from does not conceiving during their cycle are generally visible only as nonpigmented granular tissue in the ovaries for a few weeks following their formation.

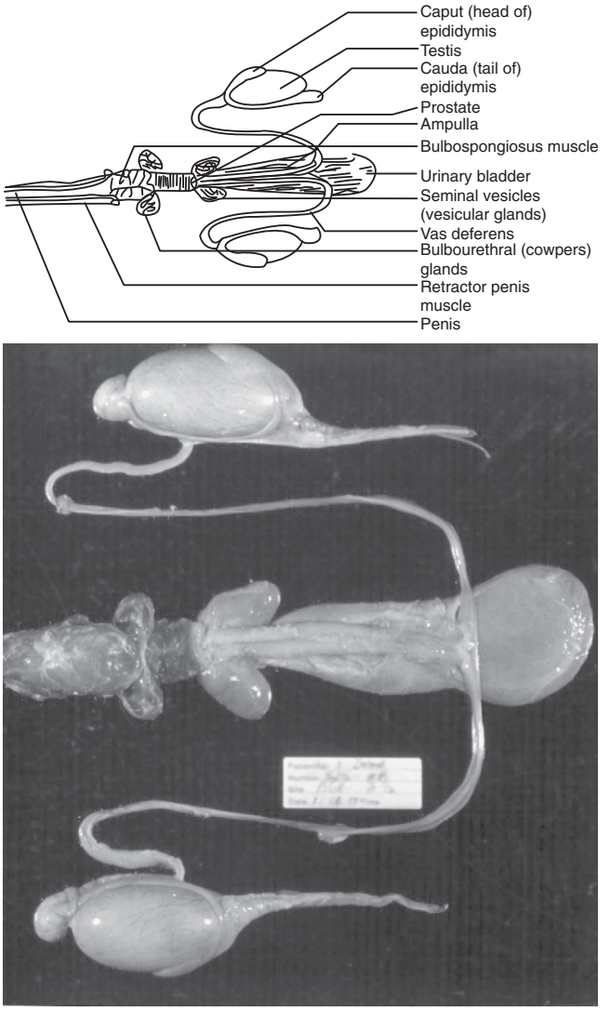

Fig. 130-1 Line drawing of reproductive tract of male white-tailed deer and photograph of the tract during November, when the rut is close to the peak.

Reproductive Timing

Breeding dates of the white-tailed deer change markedly across its range (Fig. 130-3). Initiation of the rut is photoperiod-related. This influence was illustrated most aptly by translocation of white-tailed deer from North America to New Zealand. Following translocation, reproductive activity of white-tailed deer including antler growth, velvet shedding, antler casting, and fawning all occurred about 180 days later than in the Northern Hemisphere.[7] However, white-tailed deer also are known to have an endogenous circannual clock that can regulate reproduction in the absence of photoperiod cues.[8] Genetics also is an important regulator, and different subspecies at the same latitude are known to have different rut timing. This difference can be as much as 2 months or more, and depending on the North American location, white-tailed deer have been documented to breed in

every month of the year.[9] The interrelationship of genetics and photoperiod was further shown by a study involving translocation of deer from Mississippi to Michigan and vice versa.[10] In this study, deer shifted their reproductive patterns about 3 weeks earlier or later, depending on whether in Michigan or Mississippi. However, regardless of geographic location, the rut of Mississippi deer was about 7 weeks later than that of the Michigan deer. This genetic linkage was further confirmed by the crossbred offspring of these animals which, regardless of paternal or maternal lines, had intermediate rut timing between that of the two different parent lines.

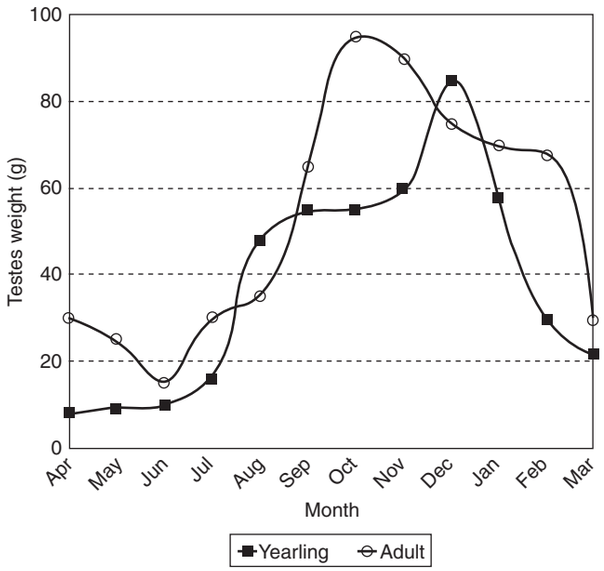

Fig. 130-2 Seasonal changes in testes weight of yearling and adult bucks. (From Griffin RN: Characteristics and management implications of the male reproductive cycle of the white-tailed deer in Mississippi. Master's thesis, Mississippi State University, 1980.)

Rut

The rut is initiated by the receptivity of the doe. However, it has been demonstrated that presence of mature males can induce earlier initiation of estrus than when does are confined with only yearling males present.[11] Adult sex ratios, which are close to 1:1, may also be important. Improvement of male-to-female sex ratios along with increased presence of mature males has shown that presence of mature males resulted in earlier breeding dates for wild populations. In two separate studies, one involving deer in South Carolina and one in Mississippi, it was demonstrated that when sex ratios of adult males to adult females was increased, the peak of rut was shifted by more than 3 weeks earlier in these populations.[12]

Estrus in the white-tailed doe normally lasts about 24 hours, and if conception does not take place, the doe will repeat the cycle at 21- to 29-day intervals.[3,13] Several copulations can occur within one estrus and multiple parenthood of the same litter can occur.[14,15] There also is evidence that a silent estrus can precede the first estrus cycle when copulation is allowed.[16]

Gestation

Gestation length normally averages about 200 days and can range from 187 to 222 days.[3] Gestation appears to be slightly longer in northern deer than southern. In Michigan, known gestation lengths averaged 199 days, whereas in Mississippi known gestation lengths of captive deer averaged 196 days.[3,17] Fetuses may develop in either or both uterine horns. The number of caruncles in the pregnant doe is variable with an average of 6 and a range of 4 to 19.[18]

The number and sex of fetuses depends on both age and nutrition. Generally, yearling does (bred as fawns) have only one fawn, 2-year-old does average 1.3 to 1.6 fawns, and prime-age does average 1.5 to 1.8 fawns.[3,9] Triplet fawns are not uncommon in well-nourished deer herds and can occur in about 3% of pregnancies, although usually one or more of the triplets have low

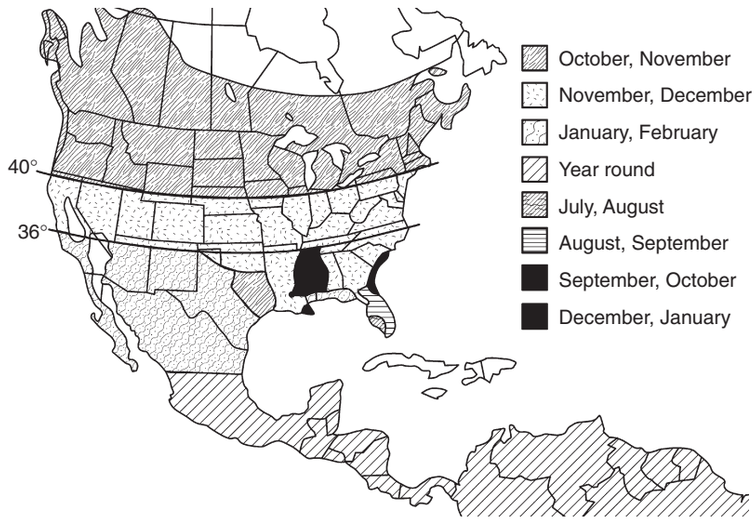

Fig. 130-3 Timing of the rut in different geographic regions of North America.

birth weights and it is unusual for all three to survive. Sex ratios favor males for yearling does and in adult does under poor nutrition, but favor females in adult does under good nutrition.[3,9] Gestation can be altered slightly by nutritional plane with does under poor nutrition having slightly longer gestation length than well-nourished does.[14] However, does with poor nutrition are also more likely to have fawns with low birth weights and to abort or abandon their fawns at birth.[19]

Parturition

Does seek seclusion just prior to parturition. Prime-age does have high fidelity to birthing sites that normally are the best fawning grounds, and consequently, they have higher survival rates for their fawns.[20] The birthing process can last up to 12 hours or more and does are known to be able to stop the process if disturbed.[2] As parturition nears, does can be seen to increase restlessness and seek out fawning cover. Does also show a peculiar gait and often hold their tails in an extended crocked position at this time.[2] Following birthing the doe usually licks her fawns dry and ingests the placenta. Twin fawns are bedded separately by the doe shortly after birth and usually remain separated until 3 or more weeks of age.[19]

REPRODUCTIVE MANAGEMENT

Genetics and Selection

Theoretically, heritable traits such as body weight, antler size, reproductive timing, and disease resistance can all be selected for through reproductive management. With wild populations this is feasible only through introduction of new animals with desirable traits or by selective removal from the breeding pool of animals with undesirable traits. For this to occur, desirable traits must be both readily identifiable and highly heritable. The most common traits selected for wild populations are those involving antler characteristics. Mature bucks have been shown to have antler heritability (h^2) estimates of from 0.03 to 0.43, with antler weight having the highest heritability, followed by antler points, beam circumference, and beam length.[10]

With captive populations selective breeding can be conducted by single sire mating and though artificial breeding techniques. Unfortunately, most traits that are of management interest have high environmental variability, and often animals may be selected for breeding because of phenotypic characteristics, which may not represent genotypic breeding value. For this reason, it is recommended that selection of breeding stock for individual traits should be on the basis of pedigree rather than phenotypic traits of individual animals.

Nutrition

Pregnant does require 155 to 160 kcal per kilogram of body weight$^{0.75}$ per day for maintenance and 131 kcal of metabolizable energy per kilogram of body weight$^{0.75}$ per day.[21] Energy requirements of does during the peak of lactation are increased about 150% of that required during gestation.[22] Protein requirements have been variously stated to be as low as 8% to 10% crude protein (dry matter basis) for body maintenance during the winter months coinciding with gestation and as approximating the growth requirements of fawns (14–22%) for lactating does.[3] Macronutrient requirements are stated as being between 0.12% and 0.29% for phosphorus, 0.64% for calcium, and 0.20% for magnesium.[23] Other nutritional requirements are not well described, but generally the most important considerations for nutrients of the white-tailed deer relate to protein and energy. Although protein and energy deficiencies have often been reported, there are few known reports of other nutrients being deficient for wild or captive white-tailed deer.

Artificial Breeding

Artificial insemination has now become fairly common for breeders of captive white-tailed deer. Vaginal insemination is routinely conducted on sedated or tranquilized does, or on does held in restraint chutes. Published data on conception success following hormone induction is lacking, although high (>65%) conception rates have been obtained with does inseminated following natural cycles and estrus detection with a vasectomized buck.[24] However, conception success following vaginal insemination of hormone-induced does rarely exceeds 40%.[17] Intrauterine insemination may result in higher conception rates than vaginal insemination, but studies on this are unknown.

Semen processing is relatively routine for white-tailed deer and conception success has been documented from both electroejaculated bucks and from postmortem collection.[24] Variation in fertility between individual bucks is unknown at present, but undoubtedly is a factor in conception rate success.

Procedures for semen processing and electroejaculation have been described.[24] Sedation with xylazine hydrochloride (2.2 mg/kg) and telazol (3.6 mg/kg) and reversal with tolazine (recommended manufacturer's dose rate) has been used successfully for electroejaculation of adult bucks.[17] Operator experience is critical and use of inappropriate sedation, ejaculation of animals with elevated respiration or hyperthermia, or use of excessive voltage can lead to stress-related death.

Although successful embryo transfer has been achieved, at present it does not appear in practical use.[25] Realistically, embryo transfer for white-tailed deer requires high cost, and the cost-to-benefit ratio is offset by high natural twinning rates and early reproductive maturity seen in this species of deer.

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CHAPTER 131

Reindeer Reproductive Management

JOHN E. BLAKE, JANICE E. ROWELL, and MILAN P. SHIPKA

Rangifer, a relatively new genus in the deer family, is indigenous to holarctic northern latitudes. Reindeer and caribou are both members of the same species, *Rangifer tarandus,* which has been subdivided into two main groups, based on antler morphology and habitat, with seven extant subspecies.[1] Reindeer and caribou can interbreed, producing reproductively viable hybrids. Nonetheless, behavioral and physiologic differences remain between the subspecies, especially in the timing of reproductive events.

Reindeer have been semidomesticated for centuries and are an economic mainstay for many native populations. Throughout most of their history they have been raised in extensive, free-ranging systems. More recently, there has been a move toward intensive reindeer farming using traditional agricultural practices, which requires a more in-depth knowledge of reproductive management.

HAREM FORMATION AND MANAGEMENT OF BREEDING REINDEER COWS

Estrous Cycles

Reindeer are seasonally polyestrous, short-day breeders, with an estrous cycle length of approximately 24 ± 3.4 days (range: 18–29 days).[2] The endocrine changes during the estrous cycle have been described in detail and are very similar to other deer species. Primiparous Norwegian reindeer exhibited considerable variation in estrous cycle length (19.4 ± 5.7 days) at the onset of the breeding season.[3] This contrasts to our work with reindeer at a similar latitude in Fairbanks, Alaska, where estrous cycle length did not vary significantly from the beginning to the end of the breeding season or among individuals. Seasonal ovarian activity among females penned without a bull is initiated in late August.[2,4] Like other ruminant species, a small transient progesterone rise, lasting between 4 and 9 days, precedes the first full-length estrous cycle of the breeding season, and reindeer may experience two or more short cycles before the onset of full-length cycles.[2] Even though an estradiol and luteinizing hormone (LH) peak may precede short cycles, these hormonal events have not been clearly linked to estrous behavior. However, the possibility that males may detect these initial endocrine changes and attempt to mount cows at this time could explain the numerous earlier reports of a 10- to 12-day cycle length.

Females left open will continue to cycle well into spring (April), having 6 to 8 estrous cycles over the winter.[2] The transition to anestrus in five adult females was characterized by either an abrupt cessation of luteal activity in April (n = 2) or the formation of a persistent corpus luteum (CL) (n = 3) beginning in late February or March. Sampling did not continue long enough to evaluate the lifespan of the prolonged CL in two cases, but from previous work, it is known that persistence into the next breeding season can occur.[4] Further investigation is needed to assess the potential effect of a persistent CL on breeding synchrony and timing of mating.

Antlers and the Onset of Estrus

Reindeer and caribou are the only members of the deer family in which females grow antlers. Although males depend on rising testosterone to harden and clean the antlers, increasing estradiol at the onset of the breeding season is responsible for female antler cleaning.[5] Anecdotal information suggests that antlers are cleaned 2 to 3 weeks before the onset of estrus, but there is no clear documentation on the interval between antler cleaning and first ovulation. Although preliminary work cannot predictably link antler cleaning with ovulation, polished antlers are a useful criterion for timing harem formation.[6]

Puberty

Recommendations generally suggest that harem cows be at least 1.5 years old or a have a body weight of at least 60 kg. Yearling and adult females initiate the first full-length cycle at approximately the same time,[2,4] suggesting that once pubertal weight is attained, age differences in body weight have little effect on the onset of breeding readiness. Both males and females can reach sexual maturity at 6 to 8 months of age, and there are numerous reports of females producing their first offspring as yearlings. However, calf pregnancies have resulted in reduced postpartum growth rate of the young females, low birth weight calves, and high offspring mortality rates. Although male reindeer can attain physiologic puberty in their first year, their subordinate position in a herd generally precludes them from breeding. If isolated with receptive females though, larger male calves can take over a dominant role and successfully breed.

Bull Introduction

In the absence of a bull, adult cows in good body condition will come into estrus, the timing of which may vary

with latitude.[2] However, introduction of a bull to a group of females before the initiation of estrous cycles significantly hastened the onset of ovarian activity by approximately 2 weeks and resulted in synchronized calving the following spring.[4]

Estrous Behavior and Detection

Behavioral estrus can be subtle, and published descriptions are inconsistent. Various authors report dilation of the preorbital glands and upright position of the tail, especially in response to touching of the perineal region. Redness and swelling of the vulva, accompanied with a mucous discharge, is not always apparent.[3] Anecdotal descriptions of cows appearing restless, searching for a bull, and mounting other cows can be found in the husbandry literature. The behavior of the male toward the female is probably the most frequently used indicator of estrus. However, there are problems with consistency and precision here, too. Once-a-day observations or observations collected only during daylight hours may not be frequent enough to detect all instances of mating.

There are few reports on the use of artificial aids for the detection of estrus. Heat mount strips and vasectomized, marked bulls have been occasionally used with variable success. We recently investigated the use of a radiotelemetric estrous detection system in reindeer. In two separate studies, successful detection of estrus and breeding was 70% ($n = 10$) and 42% ($n = 19$), respectively. The former study was the only one in which we encountered two false positive mounts, at 18 and 21 days after conception.[2] Collective radiotelemetric data indicate that individual mounts are swift, lasting 3 to 9 seconds and estrous females are mounted one to three times over a 24-hour period. The brief mount duration coupled with the low frequency of mounting most likely contributed to the poor success rate of radiotelemetric or visual estrus detection. Studies to improve mount detection with radiotelemetry are ongoing.

Estrous Synchronization and Superovulation

Interest in truncating the breeding season and artificial insemination has focused attention on synchronizing estrus. Among captive reindeer in Fairbanks, two 15-mg IM injections of $PGF_{2\alpha}$ (Lutalyse , Pharmacia and Upjohn Company, Kalamazoo, MI 49001, USA) administered 10 days apart, caused luteolysis in cycling reindeer, while a single 15-mg injection at 6 weeks after conception terminated pregnancies.[6,7] Polished antlers among a group of cows are an indication that most of the females are cycling and, hence, a reasonable cue for initiating a synchronizing protocol dependent on a normal CL. However, it does not preclude the fact that some cows may be experiencing sequential short cycles and fail to respond appropriately to prostaglandin.[7] In addition, care must be taken to ensure that the full dose is delivered into the muscle and not into the substantial subcutaneous fat. Conception following successful synchronization with $PGF_{2\alpha}$ was 88% with mounting occurring 44 to 56 hours after the end of treatment.[7]

The use of goat controlled intravaginal drug release (CIDRg: 0.33 g progesterone) is common in Europe, although very little has been published. In Finland, 8 of 10 females conceived following a 14-day treatment regimen with CIDRg devices. Mating occurred 43 hours after CIDR removal. Concurrent superovulation with follicle-stimulating hormone (FSH) resulted in an embryo recovery of only 20%.[8]

Gestation Length

Published estimates of gestation length for reindeer and caribou range from 198 to 240 days. This variability in reported gestation length may be partially explained by the limited reliability of behavioral observations of estrus and breeding, thus producing inaccurate estimates of conception. Our accumulated data, using only individuals with conception dates verified by endocrine data, provide a gestation length of 216.9 ± 6.8 days ($n = 39$). In one study, gestation length among eight captive reindeer, housed and managed identically throughout gestation, differed by 23 days and conception date was negatively correlated with gestation length ($r^2 = -0.98$, $P < 0.0001$). A Norwegian study has also reported a negative correlation between gestation length and conception date.[9] More information on the variability in gestation length and its association with calf viability and growth is clearly warranted and our current studies are addressing this phenomenon.

Calving

Pending parturition can be difficult to identify unless the reindeer are being observed on a regular basis. Udder development in reindeer is variable and may start as early as 2 weeks before parturition, although noticeable udder growth usually occurs during the last 24 hours before parturition. Like other species, reindeer females become restless during this time and will separate themselves from the herd. If there is an opportunity, she will hide to give birth. The sequences of events during parturition are similar to those described in domestic livestock and other cervidae. Calving occurs quickly, and careful observation is required to witness a birth. Normal fetal presentation is anterior with the front hooves and nose entering the birth canal. The time from visualization of a foot or nose to having a calf on the ground is anywhere from a few minutes to approximately half an hour. The cow usually lies down through parturition but may rise and lie down repeatedly. Once born, the cow will lick and may consume the membranes still clinging to the calf. A healthy calf will be standing and trying to nurse within 10 to 60 minutes. The placenta is expelled shortly after parturition, but this can take up to 2 hours. Retained placenta is rare following a normal birth but is relatively common after an abortion.

In our experience, calving difficulties in reindeer are very uncommon, but assistance is necessary if part of the calf has been visible for over 30 minutes without being born or if the cow has been straining for more than an hour with no results. Dystocia in reindeer usually results from an abnormal presentation; most commonly one leg,

or the head, is back. Posterior presentations (rear hooves presenting first in the birth canal) occur infrequently and should not necessitate assistance. We have observed a true breech position (tail and hips presenting in the birth canal with the hind legs still deep in the uterus) only with the first calf during twinning; the second twin presented in the normal anterior position. Dystocia due to an oversized calf is extremely rare, even among reindeer giving birth as yearlings.

Reindeer normally have a single offspring although twin fetuses have been found in 17.8% of harvested, free-ranging reindeer.[10] In Fairbanks, two cases of male pseudohermaphroditism have been recorded, with one occurrence being a presumed freemartin. Although confirmation via chromosome evaluation is lacking, this calf was born co-twin with a normal male. These observations suggest that twinning in reindeer is not a highly selected trait and attempts to promote it may be detrimental.

Abortion, Stillbirth, and Neonatal Death

Other than infection with *Brucella suis* biovar 4, few other infectious causes of abortion, stillbirths, or neonatal death in reindeer have been reported. However, with increasing numbers of reindeer being raised under intensive management systems and the higher number of interactions with domestic livestock, reports of infectious causes of reproductive disorders may increase. Poor feeding practices, malnutrition, and stress have been implicated in fetal and early neonatal death, but this has been poorly characterized in the literature. Accurate diagnostics are a crucial management tool, especially now that reindeer are being raised in nontraditional areas and in close contact with domestic livestock.

Early neonatal death in reindeer has also been associated with attempts to increase the prevalence of white-coated animals in Alaskan reindeer herds. Selective breeding practices have successfully generated more white-coated calves, but these calves are frequently born with multiple congenital anomalies that lead to death by predation or starvation within days of birth. These anomalies include brachygnathia, osteopetrosis, and eye malformations causing blindness.

Pregnancy Detection

The endocrinology of pregnancy has recently been described in both Norwegian[9] and Alaskan reindeer.[2] In the latter study peripheral progesterone concentrations increase immediately after conception to mean (±SE) levels of 5.89 ± 0.09 ng/ml; range 2.40 to 14.28 ng/ml (n = 10) where it remains until parturition, producing a progesterone profile consistent with species dependent on luteal progesterone throughout gestation. Although the placenta produces progesterone, this contribution is not clearly evident in the progesterone profile. Nonpregnant females will continue to cycle throughout the winter, as will cows that undergo early embryonic loss. Cyclic levels of progesterone overlap those of pregnancy throughout gestation, making peripheral progesterone a poor candidate for pregnancy detection.[2]

Plasma estradiol-17β (E_2), estrone (E_1), and estrone sulfate (E_1S) remain at baseline levels until the last 2 to 4 weeks of gestation when they increase dramatically until parturition. Significant concentrations of E_1S are not quantifiable until the final weeks of gestation, rendering the hormone of little use for pregnancy determination or as an indirect measure of placental health in the latter part of pregnancy.[2,9]

Pregnancy-specific protein B (PSPB) has been successfully used to detect pregnancy in wild caribou, extensively ranched reindeer, and captive reindeer and caribou. In both caribou and reindeer, PSPB appeared in maternal plasma 4.4 weeks (range 4–5 wks, n = 6) after mating and disappeared between 4 and 7 days following an injection of $PGF_{2\alpha}$ at 6 weeks of gestation.[6] For PSPB pregnancy determination, blood samples should be collected 6 weeks after breeding (if breeding dates are known) or after harems have been broken up.

Among captive reindeer habituated to handling and restraint, transrectal ultrasonography has been used to detect pregnancy between 35 and 60 days of gestation, although earlier detection, as in other cervidae, is probably achievable. The small size of these animals requires the use of a probe extender (a piece of slightly curved, fitted plastic pipe used to stabilize the probe and cord), thus allowing the operator to guide the probe with minimal discomfort to the animal. As in other species, rectal ultrasonography loses accuracy later in pregnancy once the gravid uterus drops over the pelvic brim and the fetus attains a position lower in the abdominal cavity. Transabdominal ultrasonography is successful only if adequate contact between the skin and the transducer can be achieved. Unfortunately this is difficult to accomplish with reindeer, even if the hair is shaved, because of air pockets within the hair shaft.

Antler retention is not always a reliable predictor of pregnancy status. Nevertheless, retention of antlers by pregnant cows has long been a technique used by wildlife biologists to assess near-term pregnancy status in wild caribou. This practice is based on the assumption that pregnant females retain their antlers until calving, or shortly thereafter, whereas nonpregnant females cast antlers 1 to 2 months earlier. It has also been suggested that well-fed, pregnant females cast antlers before calving. Neither of the above assumptions are supported by our captive animal data. In Fairbanks, where caribou and reindeer are reared together under identical conditions, 89% of the reindeer retain their antlers until after calving compared to only 32% of caribou. All that can be said with any certainty is that antler retention into mid-April can be used to infer pregnancy, although the converse is not true; a certain percentage of pregnant animals may cast before calving. Early bilateral casting, as well as asynchronous antler casting, occurs periodically in response to multiple factors related to the overall health of individual animals. This may include concurrent systemic or localized infectious disease as well as stress.

BREEDING BEHAVIOR AND MANAGEMENT OF BULLS

Rutting and Breeding Behavior

An early and very clear sign of the pending rut is the cleaning of velvet antlers. In Fairbanks, this generally

starts in mid-August with all adult male reindeer being in hard antler by the end of August. Antler cleaning in male calves is delayed until late September/early October and is not always complete. Among castrates cleaning is a protracted and incomplete event. Increasing levels of testosterone cause the antlers to harden and the velvet to split and shed, a process that can be completed within a day. The muscles of the neck thicken and the distinctive mane develops. Aggressive behavior toward other males increases, along with herding and chasing of females. Older, mature males initiate this process first, giving them a distinct advantage in establishing dominance. Males will use their antlers to threaten other males and even females during the early rut period. Rut-related behaviors of the dominant males include urination on their hind feet, a self-marking display termed tramping-urination, accompanied by distinctive vocalizations, referred to as panting or barking. Bulls will rub and scent mark shrubs and trees and thrash trees and bushes (and fences) with their antlers. Intense aggressive displays may result in the digging and marking of pits using both forelegs and antlers. Fights between equally matched males can be vicious and result in death. Male aggression directed toward uncooperative females, usually early in the season, has resulted in fatal injuries both in the wild and in captivity. In intensive farming situations where pasture space may be limited, males should be separated from females before calving and remain separated during the early rut period. This avoids potential injury to unreceptive females. It also allows managers to take full advantage of the bull effect, which requires a period of isolation between the sexes for maximum effect.[4] It is critical to recognize that males who are normally docile, friendly, and cooperative toward people and handlers become extremely dangerous and cannot be trusted until rut has ended.

Rutting Physiology

With advancing rut, voluntary food intake decreases and, at the height of the rut, males cease eating altogether.[11] Over a 77-day rut period males lost 35% of their ingesta-free mass. Of this, lean mass and body protein were depleted by 23%, whereas 78% of the fat reserve was expended with a daily energy deficit equivalent to 90% of the standard metabolic rate. This loss of mass and other physiologic changes occurred in all males 2 years of age and older, regardless of dominance status or whether or not they were penned with females This implies that all males, whether they are in bull pens or with harems, need to be carefully managed during the rut period.

The substantial energetic cost to these males puts them at higher risk of acquiring life-threatening infections during and shortly after the rut. We have observed relatively minor injuries in rutting bulls develop into extensive infections, frequently leading to septicemia. Moreover, the nature of these animals at this time makes clinical management difficult. Likewise, use of sedatives and anesthetic agents in rutting *Rangifer* during this period of physiologic change requires careful monitoring by a veterinarian. The unpredictability of anesthesia in rutting males of any species is well recognized; however, we have observed a very transient period in rutting *Rangifer* when they are extremely sensitive to the effects of cyclohexamines (ketamine and tiletamine) and alpha-2 agonists (xylazine and medetomidine). Although a combination of xylazine and ketamine is an excellent and cost-effective anesthetic agent in captive reindeer, we have encountered prolonged anesthesia (5 to 24 hours) with severe bradycardia (<30 beats/minute) and a weak pulse in rutting bulls receiving minimal anesthetic doses. A single dose of 15 mg of xylazine caused a life-threatening level of anesthesia (heart rate of 25 beats/minute) that was refractory to repeated doses of yohimbine in an adult bull that 3 weeks previously easily tolerated a dose four times that level with an additional 1 g of ketamine. Both immobilizing events occurred while the bull was in rut, providing no external means of discriminating when the animal became hypersensitive (Blake, unpublished observations). Although this case is an extreme example, it is not an isolated incident. Intensive management of rutting bulls is thus best handled by a "hands-off" approach. If handling of rutting bulls is necessary, well-constructed runways, chutes, catch pens, and crushes are vital. Movement of bulls through facilities can be accomplished using habituation techniques before the onset of rut, baiting with an estrous female and provision of catwalks.

Post-rut management requires careful dietary consideration. Some bulls come out of rut in very poor body condition and may continue to be anorexic for a short time. Careful observation is advised because these poorly conditioned animals may simply continue to starve, or equally problematic, they may suddenly gorge on available feed, which can also lead to death.

Male Dominance

Dominant males in free-ranging systems are generally between 6 and 10 years old with body size, antler size, and antler branching correlated with dominance. Reindeer husbandry practices in Norway recommend 1.5-year-old males for breeding. Because of increasing aggression with age and subsequent difficulty in handling, few herders use bulls for more than 2 or 3 seasons or older than 4 to 5 years of age. Among Alaskan reindeer producers, deantlered 1.5-year-old male reindeer have served as highly effective breeding bulls when provided exclusive access to a harem. Information on quantitative assessment of bull fertility is lacking, and bull selection is frequently based on desirable yet fairly superficial qualitative characteristics and suitable rutting behavior. As a minimum, evaluation of potential breeding bulls must include checking for descent of both testicles. Bilateral cryptorchidism occurs in reindeer and can result in an animal that behaves appropriately but is incapable of impregnating cows.

Even though removing the antlers from a dominant male can result in his losing superiority, antlers alone do not assure dominance. Age, body size, strength, and experience combine to produce dominance in deantlered males. For management purposes, antlers may be removed either while in velvet or as hard antler. However, cutting antlers close to the pedicle increases the risk of the bull acquiring head injuries which, if untreated, can lead to septicemia and death. Self-inflicted trauma to the

head is reduced if the antlers are cut above the brow tine. The brow tine appears to provide important protection to the face. Deantlered, rutting bulls are just as damaging to fences as antlered deer.

There are few studies on the optimal number of females per breeding male. The husbandry literature suggests 4 to 6 bulls per 100 cows, although some have suggested that a mature bull can cover 50 cows or more. In most captive situations, a ratio of one bull for 10 to 20 cows is the general practice. The condition of the bull during the breeding season must also be considered. A mature bull may be able to cover 20 cows, but it may be at the expense of his own condition and survival. Some management systems incorporate close observation with replacement of weakening or spent bulls with a "back-up" bull to ensure that all cows are covered.

Hormonal Control of Rut

There is abundant anecdotal information on the use of medroxyprogesterone acetate (Depo-Provera, Pharmacia and Upjohn Company, Kalamazoo, MI 49001) to reduce aggression in male reindeer. Although not approved for use in reindeer, this compound has been administered in a series of two injections (generally one in August and the second in October) in doses ranging from 200 to 400 mg per animal. In most cases it is used to reduce aggression in nonbreeding males or given to males following breeding. Anecdotal information suggests that the drug produces very tractable males who retain their antlers and go on to breed normally in subsequent seasons, but no studies have specifically addressed the impact of the drug, drug dose, or chronic use on spermatogenesis, semen quality, and fertility. The potential of the drug to help manage rutting bulls needs more thorough investigation.

Although reindeer broadly conform to many of the principles and practices of deer farming, they do represent a species that is frequently the exception to the rule. Response to the demand for reindeer-specific information is just emerging, and knowledge in this area will not only improve our ability to farm reindeer, but will add significantly to the general understanding of this species and their unique adaptations to the Arctic.

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CHAPTER 132

Reproductive Management of Axis Deer (*Axis axis*)

ANTHONY W. ENGLISH

Chital deer are one of six species of deer found in the wild in Australia.[1] There was early interest in their potential as a farmed species with the trapping of small numbers in Queensland in the early 1970s. Chital are among the most attractive of deer, and produce excellent venison, but they soon acquired a reputation as a nervous animal that was difficult to handle in captivity. There were also problems with high rates of perinatal mortality on farms, and it became apparent that there were both economic and animal welfare issues to be addressed if chital were to be successfully farmed. A program of intensive research on the species was undertaken at the Deer Research Unit, Camden, between 1985 and 1995 that resulted in the development of highly successful management strategies for this species under Australian conditions.[2] In particular, there was an opportunity to compare and contrast the needs of chital deer with those of fallow deer (*Dama dama*), a species of temperate origin and therefore a strictly seasonally polyestrous breeder with all conceptions occurring in the autumn. This is in marked contrast to chital deer, which were known to produce calves in all months of the year in herds where mating was not controlled in any way. The major thrust of the studies at Camden was to determine the factors that needed consideration in order to farm chital deer as successfully as fallow deer. Thus, studies were conducted on the biology and behavior of chital deer, including ways to minimize handling problems, on the reproductive physiology of both stags and hinds, and on carcass quality and body composition. There was development of effective programs of artificial insemination as well as ultrasonic fetal aging and pregnancy diagnosis.[3–5]

BIOLOGY AND BEHAVIOR

A major difference between chital deer and fallow deer is the temperate origins of the latter, whereas chital deer originate in India, Nepal, Bhutan, and Sri Lanka.[6] As a tropical species it was believed that their major attribute would be their ability to breed all year round, with the potential for chital hinds to produce three offspring in 27 months. This is in contrast to fallow deer that produce only one offspring annually, with strict reproductive seasonality imposed by photoperiodic variation. However, it soon became apparent that chital deer on farms in southern Australia were not realizing this high reproductive potential, partly because of the high perinatal mortality rate. Many calves born in the winter months died within a day or so of birth, especially in very inclement weather. In addition, there were often major problems when attempts were made to handle groups of chital deer on farms, given that such groups almost invariably contained all age classes, including antlered stags. With the timorous nature of the species and their often panic-stricken reaction to being closely approached, there were frequent losses from trauma and postcapture myopathy.

During the studies on chital deer at Camden it became apparent that chital deer were quite readily yarded and handled after a suitable "training and taming" process, especially once the deer were segregated into appropriate gender and age groups for management purposes.[7] Studies on the physiologic responses of chital deer to regular handling confirmed that this taming process did occur.[8] To reduce the risks of traumatic injuries during yarding, antler growth is always prevented by velvet antler removal or surgical polling. Well-trained chital deer can in fact be yarded and handled as readily as can fallow deer, through identical facilities.[2]

REPRODUCTION

It had been noted that chital hinds were capable of producing calves in every month of the year in India, Nepal, Hawaii, and Texas as well as in Australia.[6,9–11] However, when data were collected at Camden over several years it was found that there was a preponderance of calves born in the second half of the year.[3] This fitted well with observations made on the antler cycles of chital stags, with a high level of synchrony in their cycles being noted. Most stags cast their antlers in late winter and early spring, between August and October, with the subsequent development of hard antlers and other secondary sexual characteristics and a period of rutting behavior in January to June. This fitted with observations that showed that chital hinds cycle all year round if unmated, with an estrous cycle length of 19.3 ± 1.3 days and a gestation period of 234.5 ± 3.0 days. Moreover, chital hinds were known to conceive quite quickly after parturition, and it was revealed that the interval from calving to conception ranged from 18 to 118 days, with an average of 48.1 ± 27.8 days.[12] Subsequent work showed that the first detected estrus occurred at a mean time of 26.9 ± 3.0 days after parturition. Furthermore, hinds in contact with a stag in this study had a significantly shorter interval from parturition to first ovulation compared to hinds not in contact with a stag (93% compared to 43%, respectively).[13]

MATING MANAGEMENT

Intensive observation of chital stags showed that they were capable of producing viable semen even during the velvet antler phase, although the quality of the semen at that time was reduced.[4] Thus, even though a majority of conceptions occur when stags are in hard antler, there is always the potential for some hinds to conceive at other times. In herds when no mating management is practiced there are likely to be some young calves in the group at any time of the year. It was this fact that was at the heart of many of the handling difficulties that had been encountered with chital deer, with too many opportunities for misadventure when hinds and very young calves were yarded with larger groups, including adult stags. An obvious solution is to practice some form of restricted mating, to ensure that conceptions do not occur all year round. This should be routinely practiced with farmed chital deer, with births timed to coincide with optimal pasture conditions in spring, and much earlier in the pasture growing season than can ever be achieved with fallow deer. Not only does this allow the appropriate management of pregnant and lactating hinds and their young calves, with calving avoided in the winter months when perinatal mortality rate can be high, but it also offers a definite advantage in a venison production system. There is then the potential to produce prime venison at times when it may not be possible with deer of temperate origin.

In restricted mating systems that are designed to produce calves at a particular time of year it is preferable to select stags that are in hard antler, because this ensures the best possible chance of good semen quality in those animals. Routine collection of semen for evaluation would not normally be carried out on commercial farms, but it could be done if there was a need to make absolutely certain that semen quality was not likely to be a limiting factor in a breeding program. It would be prudent not to anesthetize a stag for this examination closer than 1 week to the mating commencement date.

Stag/hind ratios of 1:30 have been found to produce good conception rates but this can be varied from as low as 1:10 up to 1:50 with good results. It was found that chital stags when confronted with a number of estrous hinds were able to modify their mating behavior to cope with this situation.[3] In unrestricted, all-year-round calving groups the dominant stag is able to spend many hours tending a hind that is coming into season, until she finally accepts him. In restricted mating systems the stag will tend each estrous hind only for a short period before mating with her and moving on to another female, with good conception rates.

ENHANCED REPRODUCTION

Artificial breeding protocols developed in red deer and fallow deer have been modified for use in chital deer, with only moderate success. Artificial insemination has been quite successful, but embryo transfer has not—or at least not as successful as in red deer, but approaching the results obtained in fallow deer.

The components of an artificial insemination program are collection of semen, freezing and storage of semen, synchronization of estrus in hinds, and deposition of frozen-thawed semen in the hind. Semen can be collected from chital stags by electroejaculation, using chemical immobilization (4 mg/kg xylazine plus 4 mg/kg ketamine intramuscularly, with reversal of xylazine using yohimbine at 0.5 mg/kg IM). Chital semen is readily frozen using extenders based on TRIS-egg yolk-citrate, and thawed in a water bath for subsequent insemination.[14] Laparoscopic and cervical insemination of CIDR (controlled intravaginal drug release)-synchronized chital hinds has produced conception rates that are generally lower than for fallow deer using similar techniques, and more research is required.[4]

PERINATAL DEATH

The problem of perinatal death in calves born in winter was confirmed in studies at the DRU, Camden. There was a 48% death rate in calves born to 45 chital hinds over 4 years, with the majority of the deaths in the winter months. This compares unfavorably with a rate of 18% in fallow deer fawns at the same location.[15] The most frequent cause of calf death was found to be a complex of mismothering, fox predation, and exposure/starvation. Chital hinds are sensitive to the presence of humans when there are very young calves in the herd, and the calves are best ear-tagged at weaning, at about 3 months of age. Chital calves are somewhat smaller at birth than are fallow fawns, with females weighing 3.4 ± 0.5 kg and males 3.6 ± 0.4 kg. They can often move through gaps which will stop fallow deer fawns, which have birth weights of 3.97 ± 0.53 kg and 4.27 ± 0.70 kg, respectively.[15] This may result in mismothering and the death of calves if fences are poorly maintained.

One unforeseen cause of perinatal death in captive chital deer was the strong tendency of some adult stags to attempt to mate with hinds that were actually calving, or just after calving. When this occurs the neonate can be knocked aside and there is certainly the potential for injuries to occur. Given that a hind cannot readily escape this attention from a stag when they are in an enclosure together, it would be good management not to have adult stags in the same enclosure as hinds that are due to calve.[3]

Restricting the birth season to those times of the year when weather conditions are favorable for calf survival and when abundant high-quality pasture is available for lactating hinds has been shown to greatly reduce the problem of perinatal death in chital deer.[2]

CONCLUSION

There is no doubt that the use of chital deer on deer farms in Australia, Texas, or elsewhere can be an effective venison production system, if appropriate management strategies are put in place. Although the principles of management that have been developed for fallow deer apply equally to chital deer, some specific differences in biology and behavior do need to be taken into account when working with this beautiful species. This applies especially to differences in reproductive biology due to

the tropical origins of the chital deer, and the specific reproductive management issues that flow from this.

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CHAPTER 133

Antlers and Reproduction

MURRAY R. WOODBURY and JERRY C. HAIGH

Antlers are only indirectly involved in the outcome of the reproductive process, but they are so intimately involved with, and affected by, the sexual seasonality of cervids that it becomes essential to discuss them and the effects that sex hormones have on their growth and annual cycle.

ANTLER DEFINITIONS

Antlers are unique in the animal world and therefore the jargon used in antler discussions is also unique.

The term "velvet" refers to the skin covering a growing antler. It describes the fuzzy texture provided by many fine hairs growing on the surface. "Velvet antler" refers to the entire antler when it is in the growth phase. At this stage it is soft and lacks the mineralized characteristics of hard antler, which is nothing less than bone. Frequently "velvet" and "velvet antler" are used interchangeably to refer to the antler in its growth phase.

Antler grows from discrete areas on the frontal bone of the skull called pedicles. They are permanent features of the skull and remain after the antler is cast off in the fall as the perennial source of the regenerating antler.

The antler-pedicle junction is called the coronet, or the "burr," due to the circumferential growth of irregular knobs and pearls of antler at the junction. In first-growth antlers the coronet is less prominent, becoming more distinct with advancing maturity.

"Spikers" are yearling stags with first-growth, single-spike antlers. Spike antlers are sometimes branched, depending on the month of birth and the nutrition available for growth. Early born stags on good levels of nutrition frequently grow branched antlers in the first year. The presence of such antlers is not necessarily predictive of future antler growth.

The generic antler is composed of a major beam, with branches or tines arising from the beam. The

the tropical origins of the chital deer, and the specific reproductive management issues that flow from this.

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The generic antler is composed of a major beam, with branches or tines arising from the beam. The

Table **133-1**

Major Differences Between Antlers and Horns

Feature	Horn	Antler
Tissue of origin	Skin	Bone
Composition	Keratin	Multiple tissue types
Persistence	Permanent	Deciduous
Innervation	Lacks innervation	Highly innervated when growing
Vascularization	Nonvascular	Highly vascular when growing

number, configuration, and spatial orientation of tines is species-specific.

Antlers are often unknowingly referred to as horns. Antlers are quite distinct from horns and have several features that distinguish them from horns, including their ability to regenerate. In fact, antler is the only mammalian tissue besides the placenta that is entirely shed and subsequently regenerated. The major differences between antlers and horns are listed in Table 133-1.

ANTLER GROWTH

Most of the available information on antler growth and control deals with deer from temperate regions. The timing and regulatory mechanisms of antler cycles in tropical deer can be somewhat different than those found in temperate zones and are illustrated in Figure 133-1. However, the basic mechanisms of antlerogenesis and its regulation provided here are common to most cervids.

Antler pedicles develop from osteogenic centers on the frontal bone area. They begin growth when sexual differentiation of the fetus occurs. Testosterone is required for pedicle formation and growth. It does not directly stimulate growth, but increases the sensitivity of antlerogenic tissue to undetermined growth factors.[1] Pedicle growth is sufficient to support antler growth at about 6 to 10 months of age and is a continuous process in the first antler cycle.[2]

Hummels are antlerless red deer stags. This abnormality has been attributed to poor feeding conditions resulting in the formation of incomplete pedicles without the potential to develop normal antlers. Presumably, nutritional cues act synergistically with androgens to permit the development of this secondary sex characteristic of red deer.[2]

At the distal pedicle, mesodermal cells derived from the skin or the pedicle, or both, multiply and differentiate to make new antler tissue. At the point of growth hyperplastic fibroblasts deposit collagen, forming a well-vascularized mass on the pedicle. The new antler elongates as this growth continues in the outermost layers of the tip. Further differentiation of cells into chondroblasts and chondrocytes associated with the formation of cartilage occurs in the portions closer to the antler base. Subsequently, the antler continues to enlarge by the differentiation process at the tip and by elaboration of fibrocartilage underneath those portions. Growth is very rapid; a wapiti antler is capable of growing more than 2 cm in a 24-hour period. After a period of about 80 days a mature wapiti stag can be expected to grow immature, still uncalcified antlers in excess of 15 kg. Subsequent growth is slowed as antler maturation proceeds and full growth is achieved after approximately 120 days. Readers requiring a more detailed description of basic antler development and growth are referred to the paper by Chapman.[3]

Primary ossification of antler occurs through the process of modified endochondral ossification whereby cartilage is directly converted to bony tissue by the deposition of mineralized material within the cartilaginous matrix.[4] Chondrocytes exhibit alkaline phosphatase activity leading to the formation of trabeculae and spongy reticulum. The reticulum is then strengthened by osteoblastic activity laying down bone on the surfaces of the trabeculae (intramembranous ossification).[4] This activity eventually leads to the hardening of the entire mature antler. The calcification and ossification process proceeds up the antler from the base to the tip. The thickening of the trabeculae eventually results in the typical hard antler, consisting of thick compact bone covering a sponge-like core.

Antler metabolism is largely under the control of hormones, such as insulin-like growth factor (IGF), but antler mineralization and ossification are influenced by testosterone.[5] As photoperiod decreases the antler becomes more sensitive to the increasing presence of testosterone, resulting in completely mineralized and polished antlers at the time of rut.[6] Figure 133-1 illustrates the circannual antler growth pattern in deer from temperate zones.

Coincidental to advancing antler calcification the skin covering the antler begins to die. The exact mechanism of velvet shedding is poorly understood, but there is no doubt that vascular changes initiate the process, with velvet death due to avascular necrosis. Typically velvet shedding occurs at the same time as testosterone levels are rising. Administration of exogenous testosterone (or estrogen) will cause premature shedding of antler velvet.[6]

The hardened and cleaned antler does not die but appears to remain alive like other bones in the body. In the fallow deer a well-developed system of microscopic nutrient canals is evident, and there are living osteocytes, osteoblasts, osteoid seams, and microcallus formations indicating bone remodeling activity present until at least 3 weeks before casting.[7]

The annual loss of the antler from the pedicle in late winter or early spring is called casting. Casting is associated with diminishing testosterone levels resulting from the increasing photoperiod of spring. The line of future separation between the antler and pedicle is called the abscission line and is indicated microscopically by a narrow transverse band of minute blood vessels. Osteoclastic activity across this line is responsible for the eventual release of the antler from the pedicle. After casting the ingrowing skin-derived tissue fuses with the mesodermal tissue from the vascular channels of the pedicle,

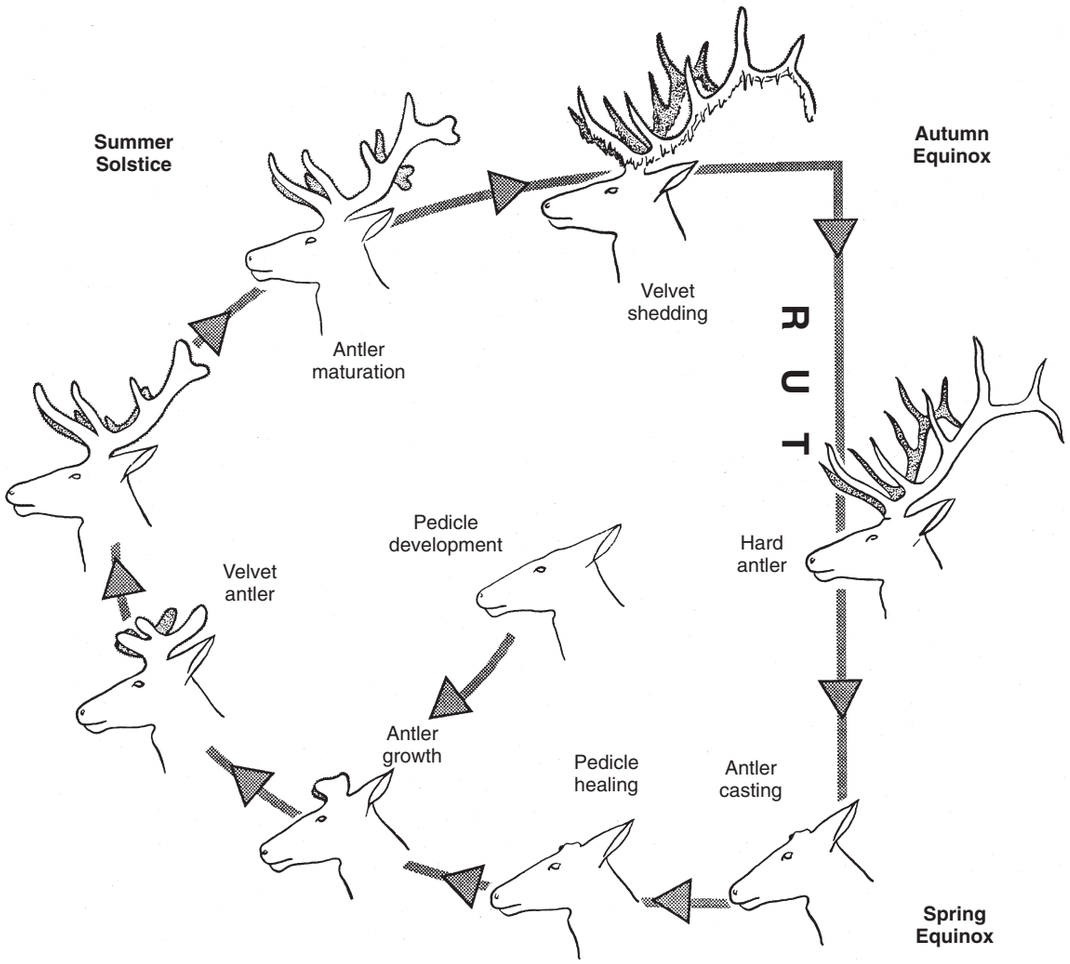

Fig. 133-1 The antler cycle. (From Haigh JC, Hudson RJ: *Farming wapiti and red deer*. St Louis: Elsevier, 1993.)

giving rise to a developing antler bud under the scab covering the pedicle.[8]

THE ANTLER CYCLE

Antler growth cycles are closely related to sexual cycles in stags and are directly attributable to variations in seasonal photoperiod influencing gonadal steroidogenic activity. The seasonal onset of reproductive activity (rut) in stags is associated with rising circulating levels of gonadotropins and consequently testosterone secretion. Readers requiring a more detailed description of neuroendocrine regulation of the antler cycle are referred to the book chapter by Bubenik.[9]

Photoperiod and Melatonin

Natural circadian rhythms of the individual are responsible for the timing of events during antler growth, but experimental evidence points to the pineal gland and its response to light as being the modulator of those mechanisms responsible for gonadotropic control and hormonal control of the antler cycle and the male and female reproductive cycle.[9] When the duration of daylight reaches a peak and the length of darkness is least the pineal gland responds by elaborating very little melatonin. As day length decreases and the period of darkness increases the pineal gland responds by secreting increasing levels of melatonin. Melatonin production is greatest when day length is decreasing to the lowest annual levels.

Gonadotropins

Rising levels of melatonin act on the pulse generator of the hypothalamus, causing the pulsatile production and release of gonadotropin-releasing hormone (GnRH). The appropriate frequency and amplitude of the GnRH pulses causes the pituitary to produce and secrete gonadotropins, meaning luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These hormones regulate the testicular output of testosterone, which is thought to ultimately control the timing of the antler cycle along with the male reproductive cycle.[10]

Testosterone

As day length decreases, testosterone secretion increases in response to rising LH levels and reaches a peak immediately prior to the rut. Thereafter, levels begin to decline until spring when a rapid decline in circulating testosterone is associated with antler casting. While testosterone levels remain low new antlers begin to grow. When antler growth nears completion the testosterone levels are once more rising. High levels of testosterone are associated with hardening and cleaning of the antler, coinciding nicely with the behavioral need for antlers during the rut.

Exogenous testosterone administration during the velvet phase results in calcification and cleaning of the antler. Administration during the hard antler phase results in retention of the antler. Therefore, high concentrations of testosterone promote calcification and velvet shedding as well as prevent casting of hard antler. Sustained high concentrations of testosterone also prevent the growth of new antlers.

The hormonal control of the antler cycle in reindeer differs somewhat from that in other cervids. In female reindeer estrogen appears to regulate the antler cycle in a manner similar to that of testosterone in males. In both sexes there appears to be an auxiliary control mechanism operative in the absence of gonads. There appears to be sufficient adrenal production of sex hormones such that antler production and regulation, although altered, can occur in ovariectomized or castrated animals.[11]

FUNCTION OF ANTLERS IN REPRODUCTION

Although students of deer behavior agree that antlers are important in intraspecific combat,[12,13] there is some debate about other roles of these structures in the life of different deer species. For instance, a role in estimation of social class and rank between individual conspecifics is suggested.[14,15] On the other hand Clutton-Brock examined five possible functions for antlers and argued that virtually their only role is for use in fights between competing males seeking access to females.[12]

Actual decisive fighting, as opposed to sparring[14,16] is likely to occur for three reasons: (1) between two closely matched stags who are competing for access to females, (2) between males that do not know one another and do not know each other's rank, and (3) in asymmetrical contests when an inexperienced young male enters the rutting ground of a prime male and fails to recognize or heed warning threats.[17]

There have been many suggestions that antlers, used as displays or advertisements of dominance among males, will permit stags to assess each other without fighting.[15,17–19] Stags appear to evaluate each other based mostly on antler size.[17,18] The length of the antler beam in elk indicates that antlers are a long distance, visual signal. The slightest head nodding is reinforced visually by movement of the elongated antlers, reinforcing the stag's presence and rank. Changes in the shape or size of the antler structure of breeding males through accidental fracture or experimental trials can lead to alterations in the stag's position in the hierarchy because such stags are immediately challenged by subordinates.[15,17,18]

There is also evidence that elk hinds in estrus will seek out the stag with the largest antlers, indicating a male-female interaction dependent on antlers in addition to the male-male interaction.[17]

Antlers also have an olfactory role in the sex life of deer. During bouts of thrash urination rutting behavior, elk and red deer spray heavily scented urine on their sideways tilted antlers.[18] The dorsal location of the urethral tip on the penis is an adaptation of elk and red deer that allows urine to be directed slightly above the horizontal as it is ejected cranially from the erect penis. The bull can scent himself on the belly, forelegs, long hairs of the neck, and the antlers.[20] Antlers are also used during the rut for making scrapes and wallows in the ground and for territorial marking by thrashing of vegetation and destruction of small trees and bushes.

Although antlers provide a visible expression of dominance, other social and individual factors are of similar importance. For example, antlerless stags will successfully defend a harem and breed. That the absence of antlers in no way inhibits the ability to breed is an important factor for the deer farmer as an antlerless stag is considerably easier to manage during the rut than one with a full head and inflicts less damage to fences, other deer, and humans.

ANTLER MANAGEMENT

Effects of Castration on Antler Growth

Testosterone promotes pedicle growth but interferes with antler growth.[9] It is required for pedicle development and the initiation of the first antler cycle. Castration prior to first antler growth results in the complete and permanent inhibition of pedicle growth. Once pedicles are developed and antler growth has been initiated, castration does not prevent antler growth. Because antler growth in the intact male occurs in the absence of significant testosterone levels, castration will not interrupt antler growth once it has begun.

Castration of postpubertal males in velvet will result in the retention of the velvet antlers without maturation. The outcome is continuously growing antlers that never mineralize, shed their covering of skin, or cast normally in the fall. Castration of animals in hard antler will result in casting of the antlers within 2 or 3 weeks, followed by the growth of abnormal antlers described earlier.

The effects of castration on antler growth and maturation can be reversed with exogenous testosterone. Weekly intramuscular 2 mg/kg injections of testosterone cypionate, enanthate, or propionate will result in mineralization of the antler and shedding of the antler skin.[21] Use caution when handling these animals as they will become aggressive and show the typical behavioral signs of rut. Testosterone injections can be stopped when the antlers are hard and polished. The subsequent abrupt fall in testosterone levels will result in shedding of the polished antlers, followed of course by the regeneration of abnormal antler growth.

Reindeer are an exception to the principles of antler growth. In this species, both males and females develop antlers soon after birth and prior to puberty. Removal of the testes or ovaries before puberty does not block growth or annual regeneration of antlers.[22] When castrated during the period of velvet antler growth, the developing male antlers are retained throughout the fall and winter but do not become overgrown. These antlers fail to fully mineralize and shed their skin, but are cast in the spring, initiating new antler growth. Reindeer antlers are renewed annually despite castration. Behavioral signs of rut in intact male reindeer can be diminished or removed by an injection of 200 to 300 mg of medroxyprogesterone acetate (Depo-Provera, Pharmacia & Upjohn).

Antler Removal

Velvet antler removal from farmed elk and deer for commercial purposes is widely practiced. Soft, growing antler harvested from elk, red deer, sika deer, and other species is processed and used in traditional Chinese medicine and as modern nutraceuticals for its benefits in treating chronic disease and tonic effect.[23] Velvet antler's reputation as an aphrodisiac is undeserved and its reported effects on human sexual function are largely organ-based rather than psychological.

Antler harvesting can be accomplished only on suitably restrained animals, especially with large cervids like elk. In the recent past chemical immobilization with general anesthetic drugs was widely used. More recently, modular handling facilities with hydraulic cradles have been developed to safely and securely hold fully awake animals for antler removal.

Modern farming practice involves the administration of local anesthesia to the nerves serving the antler and pedicle, followed by amputation of the antler just above the antler-pedicle junction.[24,25] Hemostasis is achieved by a tourniquet around the pedicle. Amputation is easily performed using any coarse saw that can be repeatedly sanitized with chemical disinfectants. Ancient methods did not involve the use of analgesia, but more modern techniques have incorporated the subcutaneous infiltration of lidocaine in a ring block at the base of the antler pedicle. Because of industry concern about chemical residues in antler products, experimental and unproven methods such as transcutaneous electrical nerve stimulation (TENS), also known as electroanesthesia, and compression of the pedicle are also used. Their effectiveness and worth have yet to be scientifically established.[26]

For safety reasons, hard antler is also commonly removed from farmed deer. Antlerless stags are easier to handle and much less dangerous to humans and herd mates during the rut. "Thrashing" behavior, using antlers as instruments of aggression on inanimate objects, often ruins fences, waterers, and hay racks. In the instance of hard antlers, anesthesia of the antler after restraint is not necessary. Animal handling and physical or chemical restraint is much easier to accomplish if antler removal is performed prior to the rut. A saw cut approximately 2 cm above the pedicle junction with a Gigli wire or fine-toothed saw will easily take the hard antler off cleanly.

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CHAPTER 134

Cervid Semen Collection and Freezing

MIKE J. BRINGANS, CLAIRE PLANTE, and JOHN POLLARD

SEASONALITY

The cervid species mentioned in this section (wapiti, red deer, white-tailed deer, fallow deer, and sika deer) are all seasonal breeders. Because of the relationship between changing daylight length and fertility, there is a limited window of opportunity to collect viable semen[1,2] (Fig. 134-1).

This window can be manipulated early via artificial lights or the use of melatonin implants. Melatonin implants have been successfully used to advance the breeding season of red deer.[1] Starting 3 months before the normal breeding season, using one implant of Regulin (Regulin, Schering Agrochemical, Ltd., NSW, Australia) per month, it was possible to obtain good quality semen 1 month before other untreated bulls of the same age.[2]

However, there were undesirable side effects. These bulls rutted early when the temperatures were hot, and then changed into a summer coat in late winter when the temperatures were cold. They also showed irregular antler growth, going through two antler cycles in 1 year.[2]

At the beginning of the breeding season, a higher percentage of secondary abnormalities can be seen in the semen sample. The timing of this varies between individual males. Similarly, individual males will display a higher percentage of secondary abnormalities toward the end of the breeding season. In some instances an excess of 50% of sperm in one ejaculate will show distal droplets before cessation of viable sperm production.[1]

METHOD OF COLLECTION

Some workers have reported collections from dummy mounting, as well as indwelling condoms in ovariectomized females treated with estrogen, but realistically electroejaculation is the most accepted method. There are two methods of collecting semen by electroejaculation.

Under Anesthetic

Initially most cervid species were collected this way.[1] However, from the late 1980s onward the method of choice to collect red deer and wapiti semen involved the use of no drugs, and a squeeze or chute.[1]

If wapiti or red deer are in full antler it may still be necessary to tranquilize or anesthetize them. Drugs used to induce lateral recumbency are listed in Table 134-1. They are either narcotic-based mixtures or a mixture of xylazine HCl and Telazol.[1,3]

Fentazine or the carfentanyl/xylazine mix is reversed with tolazoline hydrochloride (Tolazine, Lloyd Laboratories, Shenandoah, Iowa) (2–4 mg/kg, given half IM, half IV) or yohimbine hydrochloride(Antagonil, Vetrepharm, London, Ontario, Canada) (1.0–1.25 mg/kg) to reverse the xylazine, as well as Narcan (Naloxone hydrochloride injection, DuPont Pharmaceuticals, Dorval, Quebec City, Canada) to reverse the narcotic (Table 134-2). The more nervous smaller cervid species are best done under anesthesia. The drug combination of choice for white-tailed deer bucks is xylazine/Telazol mix. This is reversed with

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yohimbine or tolazoline hydrochloride (doses as previously given).

Cervids collected under anesthetic are in lateral recumbency with an assistant holding the back legs and another assistant holding the head.[1]

More electroejaculation stimulation is needed than in standing collection. Anesthetized males are more likely to spoil the collection with urine contamination or accessory sex gland contamination with this method.

A cleaner collection can be obtained by initially snaring the penis with a cotton bandage and directing it into a collection vessel, so as to avoid preputial contamination. Care is taken to collect the ejaculate in fractions, so that every collection can yield some viable sperm. If contamination from urine or accessory sex glands occurs, then that affected fraction is discarded.

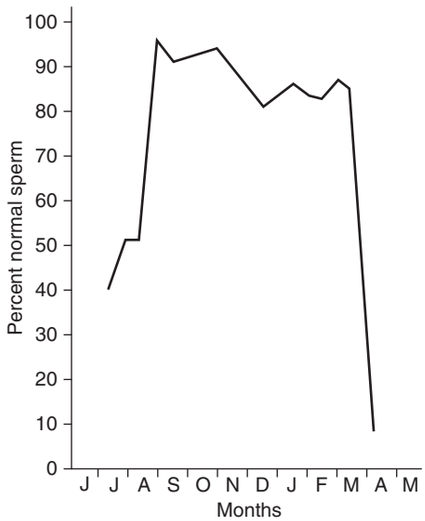

Fig. 134-1 Spermiogram of adult wapiti bulls in the northern hemisphere. (Adapted from Haigh JC, Barth AD, Cates WF, et al: Electro-ejaculation and semen evaluation of wapiti. In Fennessy PF, Drew KR: *Biology of deer production.* Wellington: The Royal Society of New Zealand, 1985, pp 197–203.)

Standing Collection

With standing collection the male is immobilized with a squeeze or crowding gate. The rectum is evacuated manually. During this process the penis can be snared with a section of gauze bandage for easier collection. The preputial area is cleaned before collection. Tranquilization may be needed in rare instances. Xylazine (30 mg total dose) can be used in red deer and wapiti. Reversing this dose is unnecessary.

EQUIPMENT

The group uses a Lane Pulsator III electroejaculator (Lane Pulsator III, Lane Manufacturing Inc., Aurora, CO) on all species. A sheep probe is ideal for the smaller species, and hand-crafted probes are used for wapiti and red deer (size 4 cm diameter for red deer and 6 cm diameter for wapiti). These can have two or three ventrally placed electrodes.[4]

Collection vessels used are normally specially blown bell-shaped glasses with a protective water jacket kept at 36° C. Thick glass-bottom shot glasses can be used in warmer climates. Standard bovine rubber collection sheaths and test tube combinations can also be used, but

Table **134-1**

Drugs Used to Induce Lateral Recumbency

Anesthetic	Wapiti (400 + kg)	Red Deer (220 kg)	White-tailed Deer (100 kg)
Fentazine*	3 ml	2 ml	
Carfentanyl* (0.06 mg/ml) and xylazine* (100 mg/ml)	2 ml mixture	1 ml mixture	
Xylazine (100 mg/ml) and tiletamine HCl (50 mg/ml) and zolazepam HCl* (50 mg/ml)			1–2 ml mixture

*Fentazine, Parnell Laboratories, Ltd., Alexandria, NSW, Australia; Wildnil (carfentanyl), Wildlife Pharmaceutical Canada, Inc., Callander, Ontario, Canada; Rompun (xylazine), Bayer, Inc., Etobicoke, Ontario, Canada; Telazol (zolazepam), Wapitiins-Sinn, Inc., Cherry Hill, NJ.

Table **134-2**

Drugs Used to Reverse Anesthetics

	Wapiti (400 kg)	Red Deer (220 kg)	White-Tailed Deer
Yohimbine (5 mg/ml)	3–5 ml	3–5 ml	3–4 ml
Tolazine hydrochloride (100 mg/ml)	3–5 ml	3–5 ml	3–4 ml
Narcan (0.4 mg/ml)	1 ml to be used in conjunction with one of the above	1 ml to be used in conjunction with one of the above	

these are more prone to extra contamination from the prepuce and abdomen. The collection vessels can be kept in a water bath or an incubator and returned quickly to either, as the fraction is collected.

TECHNIQUE

Initially begin pulsing (3 sec on, 3 sec off) at low voltage (±1 volt) and gradually build the voltage up to $2^1/_2$ volts. Normally a male will have ejaculated in a 1- to 5-minute period.[2]

With standing collections in wapiti and red deer, in most cases, it is normal to see clear seminal fluid initially. This is not normally kept to be included in the semen to be processed. This volume can be 1 ml to 10 ml. Soon after the ejaculate becomes cloudy, the white semen will be evident. After the semen rich fraction is collected it is common to see cloudy and then clear seminal fluid again. Only the semen-rich fractions (1–8 ml) are processed. Care must be taken to avoid the yellow vesicular fluid, because this is toxic to sperm (Fig. 134-2). This is more commonly seen early in the breeding season. Semen can be salvaged if inadvertently mixed with vesicular fluid, if it is quickly pipetted off the yellow viscous solution.

With both anesthetized cervids and those done standing, a second ejaculate can be obtained 5 to 10 minutes after the first. It will yield approximately the same volume and number of sperm cells. With the smaller cervid species, such as white-tailed and fallow deer, a smaller volume, higher density ejaculate is more common.

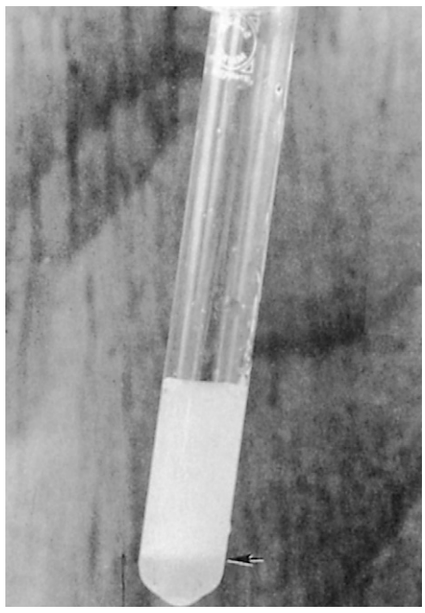

Fig. 134-2 Ejaculate in which vesicular gland secretion has been collected. The upper portion contains creamy white semen. The lower one sixth, below the separation line *(arrow)* is bright yellow. The semen should be carefully decanted before extension or freezing. Note arrow dividing line between sperm fraction (*upper*, white) and vesicular gland fraction (*lower*, yellow). (From Haigh JC, Hudson RJ: *Farming wapiti and red deer*, St Louis: Mosby, 1993.)

Extender that has been kept at the same temperature is added to the semen-rich fraction immediately, at a ratio between 1 : 1 and 1 : 3 semen/extender, depending on perception of color and density. This is pipetted into a sealed test tube and put in a water jacket (water volume 150 ml) at the same temperature as the water bath. This water jacket is placed in another container at 4° C and the semen will cool to 4° C over 1.5 hours.

The typical number of sperm cells per collection is 4000 million with red deer or wapiti, and 3500 million with whitetailed deer. The semen is placed in 0.5- or 0.25-ml straws at 30 million to 40 million sperm cells per straw for wapiti and red deer and 50 million per straw for white-tailed deer.

There are no data available on minimum doses necessary for optimal conceptions. This would have to be done on an individual male basis, as fertility varies among animals having equivalent semen quality. The lowest number of sperm cells in wapiti I have used is 300,000 live sperm per dose, which achieved a 50% conception rate in 10 wapiti cows.

The typical collection yields of 470 collections from wapiti over a 4-year period are represented in Table 134-3 and collections for white-tailed deer are in Table 134-4.

EXTENDERS

The extender used most commonly is a one-step TRIS extender with a gentamicin, tylosin, Lincospectin anti-

Table **134-3**

Semen Collection from Wapiti

Age	No. of Collections	No. of Doses*	Average†
2-year-olds	29	12–132	51
3-year-olds	21	19–229	74
Adults	420	8–248	98

*Each frozen dose contained an estimated 40 million sperm/0.25 ml straw.
†Adult red deer averaged 130 over the same period as wapiti.
Note re collection failures: 20 of 470 resulted in zero saleable semen; 11 collections were discarded because the prefreeze motility was below 70% or the post-thaw motility was below 40%; 3 were contaminated by urine; 4 were contaminated by vesicular fluid; 1 was discarded due to a high percentage of secondary abnormalities.

Table **134-4**

Semen Collection from White-Tailed Deer

Age	No. of Collections*	No. of Doses†	Average
2-year-olds	10	14–130	45
Adult	38	13–128	70

*Two of the 38 adult collections were discarded.
†Each frozen dose contained an estimated 50 million sperm/0.25 ml straw.

biotic mix (One Step Tris Extender, Gencor, Guelph, Ontario, Canada). This has been used successfully in wapiti, red deer, white-tailed deer, fallow deer, and sika deer. However, some collections of certain individual males will not freeze in such an extender with acceptable results. Other extenders can be used in these cases. For example, of 210 wapiti bulls collected, six did not freeze acceptably in the TRIS extender (Citrate extende) Two of these bulls produced good quality semen frozen in Biociphos Plus (Biociphos Plus, manufacturer IMV, France; Canadian distributor, Gencor, Guelph, Ontario, Canada) only. Triladyl (Triladyl, Minitube, Ingersoll, Ontario, Canada.) is also a good alternative extender for most cervid species, and semen from two of the "problem" bulls froze in Triladyl with acceptable results. A step A (no glycerol) and step B citrate extender can also be used. It is technically more difficult to use but is also worth trying if a bull's ejaculate fails to consistently freeze well in a one-step extender. The other two of our problem bulls had semen successfully frozen in this.

FREEZING

After cooling to 4°C, the semen is extended further to give the required dose per straw. A minimum of 4 hours after collection, it is packed into straws and frozen. The level in the vapor rack is at –140°C. The freezing box temperature is wafted up to –100°C, and the straws are placed in it. The temperature in the box will rise to –35°C and then fall at 10°C per minute to –160°C over a period of 13 minutes. The semen is then plunged into liquid nitrogen.

ANALYSIS

Each batch of semen frozen should be analyzed by thawing two straws. An immediate post-thaw motility reading is done and usually a 2-hour incubation motility reading is done after this. This gives a good indication of the percentage of live, progressively motile sperm cells in the sample. A morphologic examination is done after staining and primary and secondary abnormalities are noted.

Except for the beginning and end of normal breeding seasons, very few secondary abnormalities are seen (usually less than 1%). Of all cervid collections done, I have seen only one subfertile red stag and two subfertile wapiti males with more than 50% secondary abnormalities, at mid-breeding season. All three had histories of poor conceptions with natural matings.[2]

Most primary abnormalities seen are bent tails, usually the result of cold shock. If most of the sperm cells are affected this way it can also be from urine contamination changing the pH of the extender. A count with a hemocytometer is done to check concentration.

FREQUENCY OF COLLECTIONS

Wapiti and red deer can have their semen collected on 2 consecutive days or weekly, without any reduction in sperm numbers. Often a collection attempted on the third consecutive day will give a reduction in sperm numbers of approximately 50%. It is not recommended to collect semen on 2 consecutive days with smaller deer species, such as white-tailed deer, because of the anesthetic risk. This can routinely be done weekly without a noticeable decrease in sperm numbers.

SUCCESS OF COLLECTIONS

The success of collections varies from month to month. The peak breeding season for female wapiti in North America is early September. Some adult wapiti bulls can have their semen collected as early as the first week of August. "Pre-rut" collections generally become more successful as collections are done closer to the breeding season. For best results 2- and 3-year-old bulls are generally left until the last week of August and 1-year-old bulls are left until early September or until after the rut.

Adult bulls that are used for natural breeding generally produce less freezable semen immediately after their breeding than they do pre-rut and one or more months later. We prefer to collect semen at least 2 weeks after the bull has been removed from the cows (Table 134-5). This recommendation also applies to red deer and white-tailed deer.

"Fresh" Semen Collections and Artificial Insemination

It is routine to collect semen from wapiti, red deer, or white-tailed deer, extend the semen, cool, and inseminate immediately. I have used Triladyl, and Tris One Step extenders for this with acceptable pregnancy results[2] (Table 134-6). The part A citrate extender can also be used successfully for this.

Postmortem Semen Collection

Of eight postmortem collections done, seven gave viable sperm capable of fertilizing. It is important to handle the testicles correctly to achieve a useful collection. On the dead animal, the testicles should be pushed down into the scrotum and then the whole scrotal sac removed close to the abdomen. This is placed into a cooler at 4°C. This

Table **134-5**

Collections of Adult Wapiti Bulls, 1999–2001

Month	Average No. of Straws (40 million/straw)	No. of Collections
Aug.	96	82
Sept.	103	69
Oct.	62	25
Nov.	82	47
Dec.	75	46
Jan*.	133	12
Feb.	131	4

*Younger wapiti bulls sometimes will not produce freezable semen in January and February. Adult white-tailed deer bucks can be successfully collected until up to 1 week after antler casting.

Table **134-6**

Fresh Semen Collections and Artificial Insemination

Year	Numbers	Overall Conception (%)	Percentage of Heifers that Conceived When Inseminated with Frozen Semen*	Percentage of Heifers that Conceived When Inseminated with Fresh Semen*
1997	23	78%	70%	85%
1998	31	81%	72%	85%
1999	42	70%	60%	80%
2000	67	73%	67%	80%

*Semen at the same property over a 4-year period.

can be transported in this situation for a number of hours. The longest time frame I experienced, which still resulted in a successful collection, was 18 hours from time of death to freezing.[2]

The testicles are removed from the scrotum at the time of collection. The temperature of the tail of the epididymis is measured with a thermocouple and the extender is heated or cooled to the same temperature. The tail of the epididymis is dissected off. The first 8 cm of the epididymis is flushed with 5 ml extender, and then a syringe with 10 ml of extender is used to wash each tail (after parallel cuts are made 1 mm apart with a scalpel blade through it).

The semen/extender mix is kept at 4° C for 3 hours and then frozen. The average number of sperm cells collected with this method was 2000 million per male. The majority will have distal droplets, but of the semen collected with this method, conceptions ranged from 30% to 60%.

Complications

Of all the collections done to date we have not experienced any deaths as a result of semen collection. However, anesthetic deaths are possible in the smaller deer species, so care should be taken to monitor anesthetic depth. Preputial erosions and prolapses are common in wapiti males and may have to be resected before collection. I have also seen a rectal prolapse subsequent to semen collection that had to be repaired. At rut time this is a difficult procedure because the bugling vocalization produces intra-abdominal pressure that tends to cause a recurrence of the problem.[2]

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CHAPTER 135

Estrous Synchronization and Artificial Insemination in Domesticated Cervids

CLAIRE PLANTE, JOHN POLLARD, and MIKE J. BRINGANS

A powerful and well-respected tool for disseminating the genetics of select sires in cattle that have superior traits of economic importance is artificial insemination (AI). It is believed that this same AI technique has the potential to rapidly improve genetic gain within the cervid industry. One of the primary requirements for the success of AI with both species, however, is the identification of elite sires. In the deer industry, this is currently a limiting factor. Phenotypic characteristics such as antler size and body weight are generally reviewed and measured in assessing elite sires with the goal of improving the herd genetic makeup. The reality is that these same characteristics are governed not only by genetic makeup, but also by management techniques and nutritional condition. Standards that have been determined and tracked in cattle that reflect performance recording and analysis must likewise be developed for the cervid industry for both males and females if AI is to reach its full potential with this species.

The interest in AI in the cervid industry continues to grow as a result of the developments and refinement of reliable transcervical AI techniques in larger deer species, and minimally less invasive surgical techniques (smaller deer species) and availability of good quality frozen semen. This chapter will give a brief review of the AI techniques used in the various deer species.

In the mid-1970s, Polish scientists reported the first successful use of surgical AI of red deer.[1] In this trial, three calves were produced from 12 hinds. Likewise, successful nonsurgical AI with wapiti (*Cervus elaphus canadensis*) was reported in 1984.[2] In 1984, Haigh[3] inseminated two white-tailed deer (*Odocoileus virginianus*) with fresh-cooled semen utilizing the techniques of placing a bovine insemination pipette through a disposable vaginal speculum and depositing the semen at the os cervix. The result of this procedure was that both females delivered two live fawns. Likewise, successful surgical AI has also been reported in reindeer (*Rangifer tarandus*).[4] Today, there are reliable transcervical techniques that minimize the stress on the donors and make the AI procedures more accessible and available to everyone at a reasonable cost.

ESTROUS SYNCHRONIZATION

Owing to the seasonality of the female of the cervid species, it is standard practice to use AI early in their breeding season. The female's transition into the breeding season is characterized by silent ovulations and short-lived (8–10 days) corpora lutea, followed by the first true cycle in most deer species. The goal, therefore, is to coincide AI with the normal expected first cycle peak. General practice would follow the pattern of introducing a sire into the group 10 to 14 days after AI. This sire would be kept with the females for a minimum of at least two further cycles. The female, if not bred, will continue to cycle until such time that she conceives or the extending daylight length of late winter "switches" her cycling off. Generally, however, it is common practice for farmers to remove the male from the breeding group to avoid late calves.

The success of any AI program is to breed the female at the most ideal time for conception. In cervids, this ideal is between 18 and 36 hours after the beginning of a standing heat. The challenge in achieving this is due to the difficulties faced in trying to observe the heat signs in the female. These signs are very subtle and often occur away from observers. Unfortunately, the most successful method of heat detection (the female being mounted by the male) is impractical in a field situation. A vasectomized male that has been marked with paint or oil both under the brisket and inside his front leg may be placed with the females. However, nobody goes through this trouble. There are two signs that are most certainly difficult to determine in a field situation, but should be evaluated at the time of AI. These signs are vaginal discharge of mucus and hair rubbed off the rump (Fig. 135-1). These signs do not have to be present in each female but it does help in assessing if the overall group of females is in good standing heat.

Because of the limitations of accurately identifying standing heat in the females, it is now an accepted standard practice to program the females with a progesterone device and perform time breeding after the removal of the device (Table 135-1). The CIDR (controlled intravaginal drug release) is the most commonly used progesterone-releasing device in the deer industry. Generally, when synchronizing wapiti or red deer crosses, the CIDR-b (1.9 g progesterone) is utilized, whereas the CIDR-g (0.33 g progesterone) is being used for smaller deer. Dependent upon the species, the device is left in between 10 and 14 days and then removed manually. Once the CIDR device is withdrawn, the blood progesterone levels

A B

Fig. 135-1 Two heat signs that should be observed by the inseminator at the time of breeding: **A,** vaginal discharge of mucus and **B,** hair rubbed off the rump. These signs do not have to be present in each female, but it does help in assessing if the overall group of females is in good standing heat.

Table **135-1**

Recommendations for Estrous Synchronization in Various Deer Species

Species	Progestin Device	CIDR Duration	eCG Dose (I.U.*)	Time for AI after CIDR Removal
Wapiti	CIDR-b	12–14 days	190–200	60–66 hr
Red deer	1 or 2 CIDR-g	10–12 days	150–200	56–60 hr
Sika deer	CIDR-g	12–14 days	50	58–62 hr
Fallow deer	CIDR-g	12–14 days	0	63–67 hr
White-tailed deer	CIDR-g	12–14 days	100–150	60–65 hr
			200	50–52 hr

AI, artificial insemination; CIDR, controlled intravaginal release; eCG, equine chorionic gonadotropin.

drop, causing a luteinizing hormone (LH) surge and ovulation. Equine chorionic gonadotropin (eCG) or pregnant mare serum gonadotropin (PMSG) is often used in varying doses related to the different species, ensuring that synchronizing is tight. eCG tightens up the window of estrus by reducing the mean interval to the onset of estrus[5,6] and may reduce the spread of ovulation.[5]

ARTIFICIAL INSEMINATION PROCEDURES

Traditional transcervical insemination techniques have been utilized in the breeding of wapiti and red deer hybrids since the 1980s, and just in the last few years for red deer. Purebred hinds can be transcervically inseminated with purebred wapiti semen, and this approach can be very effective in producing hybrids.[7] To accomplish AI with these species, the use of a chute system or squeeze to restrain the female is required. This minimizes, or eliminates, the need for any tranquilization. The goal is to introduce the semen directly into the body of the uterus, just in front of the cervix. With the unusual problem females, the semen could be placed intracervically, and a pregnancy rate of 20% to 50% (depending on the semen quality) can still be achieved. Last year on 1491 transcervically inseminated wapiti females, an overall calving rate of 71.8% was achieved, with a 4.7% twinning rate. A similar calving rate of 73.5% on 950 red deer hinds was obtained, with a twinning rate of 4.1%.

Smaller deer are primarily inseminated laparoscopically. Laparoscopic AI is done with the tranquilized hind secured to a "stretcher table." The pelvic area is lifted to a 40- to 60-degree angle, and through the laparoscope, the semen is deposited at the tip of the horn, ipsilateral to the preovulatory follicle, or half the dose of semen being deposited at the tip of both horns if ovaries are not visualized. The procedure requires only minutes to do, after which the tranquilizer is reversed and the female immediately walks away. This laparoscopic technique is a less invasive technique than the traditional laparotomy; however, the technician's skills play a major role in the success of this technique. There are slight possibilities of

adhesions and scar tissue formation, which might interfere with future conception or complicate a pregnancy. The personnel required and the cost of AI is increased with this technique, and the success rate is expected to be around 70%.

Recent breeding results in white-tailed does, utilizing nonsurgical techniques, are also encouraging. White-tailed does appear to be highly fertile and some females will become pregnant if the semen is deposited at the os cervix. However, better results will be obtained if cervical intraluminal semen deposition is achieved over a short space of time. The cervix is visualized through a vaginal speculum and a bovine embryo transfer pipette is fed through the cervix, into the uterus if possible (Fig. 135-2). White-tailed does are inseminated with this technique with or without synchronization. If no synchronization is used, a teaser buck is mixed with the females. When the doe "stands," she is then inseminated. Results have been quoted as high as 80%, although they are quite variable. Last year, 35 does were inseminated 62 to 63 hours post-CIDR removal. Twenty-four females delivered at least one fawn (68.5% fawning rate), of which two carried twins, three carried triplets, and one carried quadruplets (but all died as stillborn) to term. This was the only farm that confirmed the parentage based on DNA testing. Another group has been inseminating the does at 50 to 52 hours after CIDR removal with an injection of 200 IU of eCG.[8] Over the last 2 years, this group has inseminated over 1800 does, with variable results, being from almost 0% to almost 100% fawning rate, with an overall mean of 65%. This program produces about 3% multiple births with 5 fawns and average of 2.8 fawns per doe.

Factors Affecting Success

Well-documented results are available supporting the success of AI in deer. Experienced technicians should average results around 70% pregnancy rate, with transcervical and laparoscopic techniques, with any of the deer species. The following will describe some factors influencing the AI success rate, especially related with the transcervical AI technique in wapiti and red deer. As found by other commercial AI centers, there is an extreme variation among farms (Fig. 135-3). The success rate of an AI program is affected by several factors and based on the analyzed data; some of these variations will be discussed here.

Male Effect and Semen Quality

There is great variability among wapiti bulls and stags in spermatozoal ability to maintain fertilizing capacity following thawing of frozen semen. Although there are no standards or minimum criteria used by commercial centers, it seems to be a minimum of 40% progressively motile sperm with greater than 30 million total sperm per insemination dose. Post-thawing evaluation criteria are generally done by estimating the progressive motility of the sperm. It is becoming relatively common to evaluate the longevity of motility 2 to 3 hours after thawing. It is understood that frozen-thawed semen having greater longevity tends to have better pregnancy rates when used for insemination, at least in the equine and bovine industry. Unfortunately, motility, besides being a fairly subjective measure of quality, is a very poor predictor of actual fertility.

Another important aspect affecting the quality of frozen semen is the quality of the raw ejaculate and especially the constituents of the seminal plasma at that time. Excessive seminal fluid or presence of the gel fraction has a detrimental effect on the freezability of the semen. A male will produce better quality semen for freezing if he has bred a few females earlier, or if he was collected once or twice before freezing the semen. However, at the time of thawing the semen, there is no test that can assess if the frozen semen has been compromised by excessive seminal fluid at the time of freezing.

A challenge remains to ensure proper handling and preparation of frozen semen to ensure best possible results at the time of AI. The thawing of semen is usually done at 35° to 37°C for 30 seconds. Straws should be sorted and identified only when totally submerged in

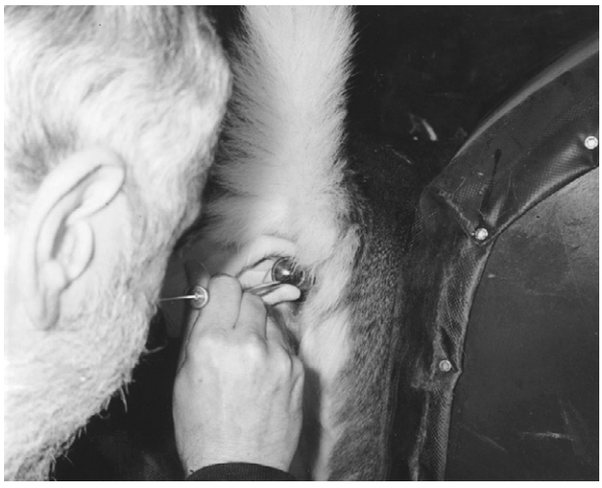

Fig. 135-2 Transcervical insemination of a white-tailed doe. The cervix is visualized through a vaginal speculum and a bovine embryo transfer pipette is fed through the cervix, into the uterus if possible.

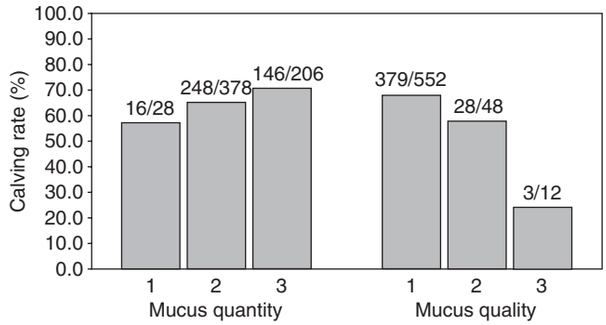

Fig. 135-3 Influence of quantity and quality of mucus at the time of artificial insemination on calving rate. This survey was performed on 612 elk cows.

liquid nitrogen. In many cases good semen is seriously damaged during handling, during shipping, and by inadequate tank maintenance.

Female Selection

Careful female selection for entry into an AI program is essential for acceptable results. The ideal females to select are the ones with a strong genetic background, calm temperament, who have produced calves every year, without calving problems and without any strange abnormalities in the pelvic area (urine poolers, etc.). These females are also in good body condition (2.5 to 3.5 BCS) and have a post-calving interval of more than 60 days. Any cow that did not calve in the previous year should not be used, nor should females that had calving problems or a dead-born calf. Females that are in poor body condition (too thin or overly fat) and calve only "every other year" should also be rejected from the AI program.

Yearling females can be used in an AI program if their weight is over 180 kg (400 lb) for a wapiti female or 70 kg (150 lb) for a red deer hind. The farm can achieve a decent pregnancy rate with AI on heifers, if the farm is successful in getting the yearling pregnant with natural cover. Usually, the yearlings enter an AI program a few weeks after the mature females, giving them a chance to have a better first cycle. First-calf heifers must be fed extra to avoid lowered pregnancy rates due to nutritional stress, and if too thin at the time of AI, they should be rejected from the program.

AI of yearling red deer hinds should be by either laparoscopic or transcervical techniques, depending upon pelvic size. An inseminator with a size 6 hand should be able to inseminate transcervically around 80% to 90% of yearling red deer hinds.

Estrous Synchronization and Quality of Heat

In any synchronization program, the application of an intravaginal device for 10 to 14 days increases the risk of local vaginal infection, which, at the worst, creates an ascending infection to the uterus, causing endometritis-pyometra. Strict hygiene must be observed at CIDR application and withdrawal. New latex gloves should be worn for each CIDR application and the applicator disinfected between each use (e.g., with Virkon). A disinfectant ointment (e.g., Hibitane cream) is also applied over the CIDR and applicator. When the CIDR devices are withdrawn, a new glove is used for the next CIDR. Care should be given at the time of injection of eCG that the female receives the full dose.

A survey was performed on 612 wapiti cows to evaluate the effect of the mucus on the success of AI. At the time of AI, the quality and amount of vaginal mucus present was evaluated and tracked to evaluate the state of the heat at time of breeding. The amount of mucus was evaluated from 1 to 3: 1, no mucus, very dry; 2, some mucus on the AI rod; and 3, mucus pouring out. The quality of mucus was also recorded: 1, clear; 2, cloudy; and 3, pus-like. A cow that was very dry at the time of AI had a 57% chance of getting pregnant, whereas a cow presenting a higher amount of mucus had 66% to 71% chance of getting pregnant (Fig. 135-3). There was a marked drop in calving rate when the females presented a cloudy/pus-like mucus at the time of breeding.

Effect of Intrauterine and Intracervical Semen Deposition

Theoretically, semen should be deposited in the uterine body, just in front of the internal cervical os. However, this task may be impossible with females with smaller cervices. At the time of breeding, the site of the semen deposition was recorded as: 0, semen deposited in the vagina, around the external cervical os; 0.75, being at least 75% down the length of the cervix; and 1, in the uterine body. On a survey performed on 413 wapiti cows, 5 of 5 cows did not get pregnant if the semen was deposited less than halfway through the cervix, and 12 of 27 cows (44.4%) got pregnant if the semen was placed 75% into the cervix; of the remaining cows, 73% (289/396) got pregnant and carried an offspring to term when inseminated into the uterine body.

Facilities

The best facility design is the one that is managed by a qualified and experienced handler who knows both the animals and the system. The handling system should allow the animals to be brought into the crush with the least amount of stress and excitement. The best "crush" for the transcervical AI is one that holds the female with a minimum of pressure. The female should not be able to "crawl" ahead in the crush and should not go down. If the crush is designed properly, a "kick-board" is not necessary, as the operator can stand to one side. The female should not be able to back out of the crush. There are a great variety of systems that can be used. Systems that allow too much movement can prove to be dangerous to the female and the technician. Inadequate restraint can result in lowered pregnancy rates, especially if the semen cannot be smoothly and properly placed immediately after thawing. Sudden movement from the female could also cause damage to the uterus while the pipette is in the uterus and cervix causing a cervical or uterine perforation, especially if the cow suddenly backs against a crush door.

CONCLUSIONS

The success of AI in cervids assures this industry future growth. With decreasing cost of semen and AI services, and a broader access to estrous synchronization supplies, the demand for AI is sure to continue and increase. Within the industry itself, herdsmen are being "pushed" to improve their herd genetics beyond what may be available within their own herd to ensure the top breeding stock is developed and maintained; otherwise, they will not be able to compete in the market.

Veterinary medicine will need to grow with this industry. There is a role with assisting the herdsman in selecting the best cows for the AI program, in overseeing the herd health and nutrition management, and coordinating the estrous synchronization program.

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CHAPTER 136

Application of Embryo Biotechnologies in Farmed Cervids

JOHN POLLARD, CLAIRE PLANTE, and MIKE J. BRINGANS

Farmed cervids represent newly adapted species to domestication possessing unique reproductive characteristics and husbandry requirements. High relative market value, demand for rapid genetic advancement, and international genetic exchange have all promoted significant interest in the development of embryo-based reproductive technologies in these species. Embryo biotechnologies have proved themselves as exceptionally valuable tools for use by progressive animal breeders in other domestic livestock species. Although a myriad of technologies currently exist by which embryos can be either created or modified, all embryo biotechnologies applied to the production of living progeny must end in the transfer of embryos to suitable recipients. The basic processes of embryo recovery, manipulation, and transfer referred to collectively as embryo transfer, brings with it powerful production and clinical benefits related to maximizing female reproductive capacity, advancing the rate of genetic progress through shortening of generation intervals, lessening the cost of genetic transport and threat of disease transfer, as well as the intentional elimination of specific environmental pathogens from infected donor populations. The adaptation of embryo biotechnologies for use in farmed cervids has been

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Application of Embryo Biotechnologies in Farmed Cervids

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Farmed cervids represent newly adapted species to domestication possessing unique reproductive characteristics and husbandry requirements. High relative market value, demand for rapid genetic advancement, and international genetic exchange have all promoted significant interest in the development of embryo-based reproductive technologies in these species. Embryo biotechnologies have proved themselves as exceptionally valuable tools for use by progressive animal breeders in other domestic livestock species. Although a myriad of technologies currently exist by which embryos can be either created or modified, all embryo biotechnologies applied to the production of living progeny must end in the transfer of embryos to suitable recipients. The basic processes of embryo recovery, manipulation, and transfer referred to collectively as embryo transfer, brings with it powerful production and clinical benefits related to maximizing female reproductive capacity, advancing the rate of genetic progress through shortening of generation intervals, lessening the cost of genetic transport and threat of disease transfer, as well as the intentional elimination of specific environmental pathogens from infected donor populations. The adaptation of embryo biotechnologies for use in farmed cervids has been

ongoing for a number of decades. Recent advances in transcervical artificial insemination, embryo recovery, and embryo transfer technologies in red deer, as well as the development of in vitro embryo production systems, is continuing the clinical development of embryo transfer in cervid species. This chapter will describe the current status of embryo biotechnology development and use in farmed red deer and wapiti (North American elk) and give effective clinical methodologies where available.

CERVID BIOLOGY, REPRODUCTION, AND EMBRYOLOGY

Collectively, cervids have experienced a long and complex association with humans, inspiring study and pursuit as a primary food source over the millennia. Only in recent history have we attempted to domesticate and selectively breed a limited number of cervid species for agricultural purposes. Chief among those selected for domestication have been members of the *Cervus elaphus* family. Members of the *Cervus elaphus* family, including European red deer (*Cervus elaphus elaphus*), North American elk (*Cervus elaphus nelsoni*, *manitobensis*, and *roosevelti*), and Siberian elk (*Cervus elaphus siberi*), while broadly spread both in terms of their geographic distribution and phenotypic appearance, interbreed readily, producing fertile hybrids. Wapiti and red deer exhibit a seasonally restricted polyestrous form of reproduction in which unmated females ovulate a single oocyte at intervals averaging 18 days in red deer and 21 days in wapiti (see Chapter 125). Wapiti and red deer have additionally evolved naturally to calve in sequence with the flush of grass growth in the spring. Calving in conjunction with spring grass growth requires successful conception to occur in September-October in North America, as gestation lengths average 233 days in red deer and 245 days in wapiti. A return to cyclicity in mature females and the initiation of cyclicity in yearlings occurs in response to decreasing daylight length. In North America, wapiti generally begin to exhibit fertile estrous cycles in the middle of September, while red deer hinds begin to cycle in early October. In unmated females, ovulations may continue to occur through March or April. Such a period of extended cyclicity rarely occurs, however, as the high natural fertility exhibited in wapiti and red deer receiving adequate nutritional support generally leads to a high percentage of females conceiving (>85%) on their first estrous cycle. In both wild and farmed wapiti and red deer, breeding activity is thus concentrated to a very restricted period in the early fall.

Embryonic development in wapiti and red deer takes place in the oviduct and the uterus. The first, second, third, fourth, and often fifth embryonic cell cycles transpire within the lumen of the oviduct in close association with the mucosal epithelium. Wapiti and red deer embryos appear to first enter the uterine lumen approximately 6 days following ovulation. Coincident or just prior to their entrance into the uterus, wapiti and red deer embryos undergo a process of cellular compaction during the fifth cell cycle, which leads to development of distinct inner and outer cell groups. Compaction is followed by the formation of the fluid-filled cavity known as the blastocelic cavity within the embryo. Formation of this blastocelic cavity coincides with the initial differentiation of epithelial cells from pluripotent embryonic cells and denotes transformation from the compact morula to blastocyst stage. Cellular differentiation and cavitation occur around 6.5 days after ovulation in both red deer and wapiti. Wapiti and red deer embryos are generally darker in color than bovine or ovine embryos at both the compact morula and blastocyst stages. Hatching of blastocyst stage embryos can occur as early as 7.5 days following ovulation, with elongation occurring between days 13 and 16.[1] Elongated red deer blastocysts signal their presence to the maternal system through the secretion of antiluteolytic interferon between days 16 and 22.[2] Attachment of blastocysts occurs by day 30 of gestation.[1] Fetal growth in wapiti and red deer is linear between days 30 and 50 of gestation with significant differences in both fetal size and placentome development at each of these gestational time points readily apparent by ultrasound examination.[3] Cervid artificial breeding programs involving group synchronization make use of these differences by delaying the introduction of backup bulls or stags for at least 10 days following artificial insemination (AI) or 4 days following the transfer of embryos so as to clearly distinguish between artificially and naturally sired conceptions.

APPLICATION OF ARTIFICIAL BREEDING TECHNOLOGIES IN WAPITI AND RED DEER

The wapiti and red deer breeding industries have been expeditious in their acceptance of reproductive biotechnologies. This rapid uptake of reproductive technology use has been encouraged by inherent factors unique to this industry, including a high level of natural fertility in red deer and wapiti, the co-advent of commercial wapiti and red deer farming with the evolution of commercially useful reproductive biotechnologies, as well as the ubiquitous pursuit of genetic advancement by breeders and the resulting competitive nature of the respective industries. The principal reproductive technology in clinical use within this species, as with other livestock species, is artificial insemination (see Chapter 134). Unique to the wapiti and red deer industries, however, is the universal use of a single timed insemination of cryopreserved semen at the beginning of the breeding season. As a carryover from their recent adaptation from the wild state, reproductive function in red deer hinds and wapiti cows suffers significantly from excessive handling. A single timed insemination allows for a reduction in handling stress to a minimum and the simultaneous concentration of management resources. The remarkable natural fertility of this species has permitted the evolution of a breeding system in which the timed insemination of a single straw of cryopreserved semen results in mean calving rates above 75%. The evolution of a timed single insemination system in wapiti and red deer has proved itself as an invaluable asset to the pursuit of genetic advancement and will serve as a model system for the development of all future reproductive technologies in this species. Of particular interest to the wapiti breeding industry is the

production and clinical concern related to the successful covering of cows as early in the breeding season as possible. Though, as discussed previously, wapiti cows may exhibit fertile estrous cycles from September through April if left unmated, the natural condition of this species is for a very high percentage of wapiti cows to conceive at their first ovulation if covered successfully. Wapiti breeders generally wish to mimic this natural pattern of reproduction as closely as possible in farmed animals, as the value of calves is at least partially determined by their birth dates, with late-born calves (calves born after June) severely discounted. A further physiologic complication, adding to the restricted breeding season, is the rapid accumulation of internal fat that can occur in wapiti cows if left unbred. Prolonged intervals between weaning and breeding can lead to excessive deposition of pelvic fat that can negatively impact both the ability of cows to be artificially inseminated and dystocia rates at parturition. These combined factors are of such importance to the industry that all reproductive technologies applied to this species must be designed to be implemented in the 5-week period beginning in the middle of September. In the case of embryo-based biotechnologies, this concentration of breeding activity has acted to severely restrict the time available to the practitioner for donor manipulation, as breeders generally wish for donors themselves to conceive successfully within this same 5-week period. This has proved a most challenging restriction, as the hormonal manipulation of donors in preparation for embryo production often proves stressful and disruptive to donor cyclicity over an extended period following treatment. Reproductive technologies intended for use in cervids must thus be designed from their onset to be both limited in treatment duration and minimally invasive so as not to disrupt normal estrous cyclicity in the manipulated females immediately following treatment.

Embryo Biotechnologies

Any discussion pertaining to the application of embryo biotechnologies to farmed cervids must begin with appropriate recognition given to this species group for the pivotal role it has played in the study of mammalian embryology. In 1651, William Harvey, following careful dissection of reproductive tracts obtained from red deer hinds harvested by King Charles I, produced evidence that mammalian gametes and embryos exist in a free and unattached state during the first weeks of life. The recognition of this developmental period in red deer, during which embryos remain unattached and therefore available for recovery, manipulation, and transfer, was critical to the advent of embryo biotechnologies in mammals. Developing embryo biotechnologies such as embryo transfer, embryo cryopreservation, in vitro embryo production, intracytoplasmic sperm injection, and adult animal cloning have the potential to dramatically alter the very manner in which we view our own reproductive capacity and that of the animals under our care.

Prehatching stage embryos possess several unique characteristics that make them effective for use in advancing genetic change and health-related biosecurity. Chief among these beneficial characteristics are that they are very small (averaging ~150 μm in diameter) and are encased within an impermeable protective shell. This glycoprotein shell, referred to as the zona pellucida, is equivalent to the shell on the familiar chicken egg, in that it completely encases the developing embryo and is impermeable to bacterial and viral pathogens. Because of the impermeability of the zone pellucida, zona intact embryos can be washed free of contaminating pathogens and thereafter be certified free of disease.[4] While it is confined within the zona pellucida, mammalian embryos remain very small (~150 μm in diameter) and are therefore very economical to transport to distant locations. It is the significant size advantage of the embryo that in fact grants embryo transfer systems their principal economic advantage over conventional live animal transport systems. Many hundreds and even thousands of embryos can be transported for less than the cost of a single airline ticket. An entire herd of deer or wapiti could be shipped using these methods to any location across the globe. These combined economic and health advantages make embryo transfer a logical choice for the safe international exchange of cervid genetics.

Embryo transfer is the process by which embryos are recovered from one female (the donor) prior to their attachment to her uterus, and thereafter transferred to the reproductive tract of another female (the recipient) for full-term development to live offspring. During the process of embryo transfer, there remains full genetic linkage between the donor female and the embryos that she produces for transfer and the progeny ultimately produced by the recipients. In contrast, there is no genetic relationship established, at all, between the recipient and the offspring as a result of embryo transfer, though there is a significant health status relationship established in utero between the recipient and the progeny of the embryo transfer donor. The success of embryo transfer (pregnancy to term) is dependent upon the recovery of embryos from the donor and the proper interaction between the transferred embryo and the recipient's reproductive tract. This interaction depends on the health of the transferred embryo, the health of the recipient's reproductive system, and their synchrony at the time of transfer.

The potential power of embryo transfer within cervid production rests within two technologic pivot points on which both the genetic makeup and health status of selected animals can be manipulated. The first pivot point exists within the relationship between the biologic donor and the embryos that she produces. Within this relationship, while there is full genetic linkage between the biologic dam and her embryos, there is no health status relationship. By this we mean that although embryos inherit all their genes from their sire and dam, they inherit none of their biologic parents' contaminating pathogens over the first week of life. The second pivot point exists within the relationship between the recipient female and the embryo transfer derived progeny that she produces. In this relationship, there is no genetic relationship between the recipient and the transferred implanted embryo offspring, but there is complete (or at least partial) health status linkage. This implies that by intentionally selecting recipients of a specific health

status, the breeder may thereby select the desired health status of the offspring she will produce. By putting these two pivot points together within an integrated production system, embryo transfer permits cervid breeders to intentionally, sustainably, and simultaneously manipulate both the genetic composition and health status of animals under selection.

Three principal streams of embryo-based reproductive technologies exist today—multiple ovulation and embryo transfer (MOET) systems, in vitro embryo production (IVP) systems, and somatic cell–based cloning systems. Only MOET and IVP systems will be addressed here, as somatic cell–based cloning systems have not yet evolved to a clinically productive state. MOET and IVP production streams differ only in the manner in which embryos are derived from donor females. In MOET systems, in vivo derived embryos are directly recovered from superovulated donor females following fertilization and before hatching from the zona pellucida. In IVP systems, mature or immature oocytes are recovered from donor females. Recovered oocytes are then fertilized in the laboratory with resulting in vitro derived embryos cultured to a transferable stage of development. Following embryo recovery or production both MOET and IVP systems utilize similar embryo cryopreservation and transfer methods.

Multiple Ovulation and Embryo Transfer Systems

Multiple ovulation and embryo transfer systems involve a three-step process for the in vivo production and recovery of embryos. The initial step in the MOET process involves the synchronization and superovulation of donor females. Superovulation is followed by the breeding of donor females (either by natural cover or artificial insemination) to allow for in vivo fertilization of oocytes. The final step of in vivo embryo production encompasses the recovery of embryos from the donor's uterus or oviducts. MOET has been applied to both red deer and wapiti over the past two decades. Even though red deer and wapiti are part of the same genetic family (*Cervus elaphus*) their response to MOET treatment, and particularly their response to gonadotropin-stimulated superovulation, differs significantly.[5] Superovulation is the process by which females are stimulated with exogenous gonadotropins to increase their ovulation rate above that normally occurring in the species under investigation. During a typical estrous cycle both red deer and wapiti cows normally ovulate only a single oocyte. Superovulation in these species would thus be defined as any treatment that would increase the ovulation rate above a single oocyte. A limited number of experimental studies have addressed superovulation in either red deer hinds or wapiti cows. These studies have demonstrated that while red deer will respond at moderate rates to superovulation treatment with commercially available purified gonadotropin, wapiti cows do not.[5,6] This difference is intriguing because red deer and wapiti interbreed readily, have very similar reproductive patterns, and are considered to be members of the same species.[6] Although earlier attempts to superovulate wapiti utilizing impure preparations of follicle-stimulating hormone (FSH) and miniosmotic pumps were at times successful,[6,7] more recent attempts involving highly purified FSH preparations currently available have met with failure. Wapiti cows treated with purified FSH preparations appear not to respond to exogenous gonadotropin either in the growth of supernumerary follicles or in the ovulation of multiple oocytes. The nonresponsiveness of wapiti to superovulatory treatments may be based either in the biologic incompatibility of commercially available gonadotropin hormones with wapiti FSH receptors, or in yet undiscovered powerful intraovarian control mechanisms that inhibit simultaneous growth in follicular cohorts to ovulatory stages in mature wapiti. Whatever the underlying mechanism, the inability to superovulate wapiti cows has been the primary limiting factor in the development of MOET in farmed wapiti. Of interest to the application of advanced embryo biotechnologies in wapiti is our recent finding that juvenile wapiti donor calves (3- to 4-month-old calves) are responsive to gonadotropin treatment, averaging 7.3 and 15.7 cumulus-oocyte complexes recovered per control and superstimulated wapiti calf, respectively.

In red deer, early superovulation protocols included equine chorionic gonadotropin (eCG) within the treatment regimen, and more modern methodologies have made use of highly purified FSH alone (purified ovine FSH [oFSH, Ovagen, ICP, Inc., Auckland, NZ] has become the commercial gonadotropin of choice in red deer). A synchronization and superovulation protocol that has been extensively tested by our clinical group in the commercial production of red deer embryos in presented in Table 136-1. In brief, the first controlled intravaginal drug release (CIDR-G; Interag, Hamilton, NZ) device is inserted into the vagina of each donor hind on day 0 of treatment. On the ninth day of treatment the initial CIDR-G is replaced by a new CIDR-G in conjunction with the initiation of FSH treatment. The total FSH dosage used for superovulation in red deer is dependent upon the genetic background of the donors in question. English strains are generally very responsive to gonadotropin stimulation, but eastern European strains are not. For English strains a total dose of 0.4 to 0.5 unit Ovagen is commonly

Table **136-1**

Superovulation Protocol for Red Deer Hinds Utilizing a Single Timed Transcervical Insemination

Treatment Day	Treatment
Day 0	Insert CIDR-G
Day 9	Replace CIDR-G; AM and PM injections of oFSH*
Day 10	AM and PM injections of oFSH
Day 11	CIDR-G removal; AM and PM injections of oFSH
Day 12	AM and PM injections of oFSH
Day 13	Artificial insemination at 48 h after CIDR removal
Day 20	Embryos flushed 228 h after CIDR removal

*Ovagen (total dosage dependent upon genetic strain).
CIDR, controlled intravaginal drug release; FSH, follicle-stimulating hormone.

prescribed, but a full 1.0 unit of oFSH (Ovagen) may be required for German, Croatian, or Romanian lines. Regardless of the strain in question, FSH is administered to each donor in eight equal doses or in a declining dose regimen. Injections are administered at 12-hour intervals over a 4-day period. On day 11 of treatment, the second CIDR-G is removed in conjunction with the administration of the sixth FSH injection. Following the final injection of FSH on day 12, donor hinds either are placed with selected stags for natural cover or are artificially inseminated. Although natural cover provides some advantages in terms of maximizing sperm number and viability, it is disadvantaged by the limited selection intensity afforded by resident stags on individual farms, by the number of hinds that can be covered by an individual stag, and by the tendency of red deer stags to focus their attention on individual favored hinds while leaving other unfavored hinds unbred. Alternatively, superovulated red deer hinds may be artificially inseminated using either laparoscopic or transcervical methods. The use of artificial insemination affords significant benefits to breeders, including increased intensity and variety of stag selection, the ability to breed large numbers of hinds to individual stags, and the capacity to assure semen deposition in the uterus of each treated hind. Although laparoscopic insemination has been the traditional technique of choice in red deer (and may still be necessary for smaller yearling hinds), highly effective transcervical insemination techniques have recently been developed in red deer (see Chapter 134).[5] In previous studies, laparoscopic insemination of superovulated hinds was found to decrease both fertilization and transferable embryo recovery rates when compared to naturally covered hinds. In contrast, we have found a single timed (48 hours after CIDR removal) transcervical insemination to be highly effective. Table 136-2 presents embryo production data from red deer donors superovulated utilizing the protocol presented in Table 136-1 and inseminated transcervically with a single dose of cryopreserved red deer semen 48 hours after removal of the second CIDR device. A single timed transcervical insemination allows for a minimum of donor handling and does not require either general anesthesia or abdominal invasion. Overall, fertilization was observed in 89% of the transcervically inseminated superovulated donors with embryo production averaging 4.6 transferable embryos per donor.

Embryos may be recovered from red deer hinds utilizing either surgical or transcervical flushing methodologies and commercial media. Surgical flushing has been effectively employed in red deer for several decades and remains the procedure of choice for use with small yearling hinds or in programs that demand oviductal stage embryos. Transcervical flushing methods have now been developed for red deer, which are equally efficacious to surgical procedures (Table 136-3). Transcervical procedures may soon displace surgical methods as they are significantly less stressful on donor animals and do not require general anesthetics.

The surgical recovery of red deer embryos is accomplished using methods similar to those used for other small domestic ruminants. Surgical flushing is conducted under general anesthesia (water and feed are removed from donor hinds for at least 12 hours prior to surgery) with the reproductive tract exteriorized through a midventral laparotomy. A very effective general anesthetic protocol that has proved to be exceptionally safe for use with both red deer and wapiti is the mixture of xylazine (Bayer AG, Germany) and carfentanil citrate (Wildife Pharmaceuticals, Inc., Fort Collins, CO, US).[6] The mixture of xylazine and carfentanil is administered as an intramuscular injection and is reversed by an intravascular injection of tolazine hydrochloride (Lloyd Laboratories, Shenandoah, IA) and naltrexone hydrochloride (Wildlife Pharmaceuticals, Inc., Fort Collins, CO, US). Once exteriorized, the lumen of each uterine horn is cannulized using a silicon molded Foley catheter (12 F for yearling hinds; 14 F for mature hinds). The catheter is

Table **136-2**

In Vivo Embryo Production in Superovulated Red Deer Hinds Bred by a Single Timed Transcervical Insemination

Factor	Number and Response
Total number of donors	51
Total number of donors responding*	45
Total number of donors with fertilized embryos	40
Total number of recovered embryos	346
Mean number of embryos recovered per donor	7.68
Total number of transferable embryos† recovered	209
Mean number of transferable embryos† recovered per donor	4.64

*Donors with 1 or more ovulations.
†Grade 1 morulae and blastocysts.

Table **136-3**

Comparison of Embryo Recovery Rates Following Either Surgical or Transcervical Flushing of Superovulated Red Deer Hinds

Feature	Surgical Recovery	Transcervical Recovery
Total number of donors	19	26
Total number of recovered embryos	144	202
Mean number of embryos recovered per donor	7.58	7.77
Total number of transferable embryos* recovered	100	109
Mean number of transferable embryos* recovered per donor	5.26	4.19
Total number of corpora lutea†	197	252
Recovery rate (%)	73.1	80.2

*Grade 1 morulae and blastocysts
†Corpora lutea number determined by visual observation in surgical recoveries and by rectal palpation in nonsurgical recoveries.

inserted through a blunt puncture made at the base of each uterine horn with the catheter's balloon subsequently inflated with 4 to 7 ml of air or saline. The endometrium of red deer is quite friable and great care must be taken not to split or damage this tissue during either balloon inflation or medium infusion. With the Foley catheter secured, the infundibulary os is cannulated using a tom-cat catheter attached to a 10-ml syringe. Medium is then gently flushed through the length of the oviduct into the uterus and out the Foley catheter into a collecting dish. As the red deer oviduct is a rather delicate structure, flush medium should be gently pulsed through the tom-cat catheter so as not to rupture the ampullary wall. After the oviduct is flushed with 10 ml of medium, a blunted 20-gauge needle is passed through the uterine wall just caudal to the uterotubal junction. Another 40 ml of medium is then flushed through the cannulated uterine horn. The entire procedure is repeated on the contralateral oviduct and uterine horn. Puncture sites where Foley catheters have been passed through the uterine wall must be secured with inverting sutures, or endometritis may result. The exteriorized reproductive tract should be continually irrigated with warmed saline to reduce the incidence of surgically induced adhesions.

Nonsurgical embryo recovery was initially developed and tested by our working group during the 1999 breeding season. The evolved procedure is identical to transcervical methods ubiquitously used in bovine embryo recovery, save for the size and composition of the flushing catheter and requisite size of the operator's hands. Essential to the recent development of transcervical methods for artificial insemination, embryo recovery, and embryo transfer in red deer was the entrance of highly creative female clinicians possessing diminutive hands talented in palpation. Nonsurgical embryo recovery in red deer is accomplished using a 14-gauge metal flushing catheter passed transcervically into the uterine lumen under the guidance of rectal palpation. After the catheter's balloon is carefully inflated at the base of the respective uterine horn, flush fluid is injected and then withdrawn from the uterine lumen in a manner similar to that used in cattle. A total of 500 ml of medium is flushed per uterine horn. An epidural anesthetic is used to facilitate passage of the flushing catheter through the cervix. As transcervical procedures access the uterine lumen alone, only embryos at or beyond the compact morula stage can be recovered by these procedures. This is generally adequate for commercial embryo recovery programs, however, as compact morulae and blastocysts are the embryonic stages of choice for cryopreservation and transfer. In direct comparisons, transcervical embryo recovery methods have been shown to be as effective and productive as traditional surgical procedures (see Table 136-3) when flushing 9.5 days following CIDR device removal.

Although wapiti cows do not respond to currently available superovulation procedures, individual embryos can be recovered from donors synchronized as for artificial insemination. Such donors will ovulate only a single oocyte, but clinical experience has demonstrated that individual embryos may be collected from a high percentage of the donors (~70%) if flushed 9.5 days following CIDR device withdrawal. Bovine methodologies and equipment for transcervical uterine flushing can be directly applied to wapiti cows, as they are approximately equivalent in size and anatomic configuration.

Methods for the in vivo production of embryos in fallow deer have also been developed.[8] Embryo production from MOET technologies in fallow deer appear intermediate to that of red deer and wapiti. Although fallow deer appear more responsive than wapiti to superovulation, the production of embryos following gonadotropin treatment falls significantly short of that obtained in red deer. Embryo recovery rates have averaged 2.1 embryos per superovulated doe.[8] Superovulation protocols have often included the addition of a low dose of eCG (100–200 IU) to the purified oFSH treatment. Fallow does may be laparoscopically inseminated using a single dose of semen, with embryos recovered surgically using procedures similar to that described previously for red deer. Recovered embryos are transferred to synchronized does using the laparoscopically based minilaparotomy method. Pregnancy rates following the transfer of fresh embryos in fallow deer have been similar to those obtained in red deer. Transcervical embryo recovery and transfer methods have not been developed to clinically productive states in fallow deer due to their diminutive size.

In Vitro Embryo Production

In vitro embryo production technologies allow for the creation of embryos in the laboratory from gametes gathered from either fertile or infertile donors. In vitro embryo production (IVP) is a generalized term referring to a number of associated technologies, including those associated with sperm and oocyte recovery from donors, in vitro oocyte maturation, in vitro fertilization, and the in vitro culture of embryos. In vitro embryo production technologies are among the most interesting and powerful of all the new reproductive technologies, as they empower the practitioner to circumvent infertility. Such infertility may be related to anatomic, pathologic, or human-induced causes in adult animals, or may be related to reproductive incapacity normally present in juvenile animals not yet achieving the age of puberty. Through IVP systems, juvenile donors may be induced to reproduce following the transfer of IVP-derived embryos to suitable mature recipients. Artificial breeding programs utilizing juvenile donors have the capacity to significantly increase the rate of genetic improvement by decreasing the generation interval with the selection program.

The process of IVP begins with the acquisition of gametes from both the dam and sire of interest. In both red deer and wapiti, sperm from desired sires is available in a cryopreserved state. This is advantageous because cryopreserved sperm are always available and the freezing and thawing process facilitates the initiation of in vitro capacitation. Over the past decade, significant progress has been made in the technologies of oocyte recovery from living donors. The technique selected depends upon the donor's species and age. Laparoscopically guided oocyte aspiration can be used for the recov-

ery of oocytes from either adult or juvenile donor females. Transvaginal oocyte recovery techniques utilizing rectal palpation have also been developed for use in mature red deer and wapiti females, by which either immature or mature oocytes may be recovered. Transvaginal oocyte recovery may be conducted under ultrasound guidance or under the guidance of rectal palpation alone (referred to as blind aspiration). Donors in intensive oocyte recovery programs can be repeatedly aspirated over several months without apparent harm or long-term adverse effect on the donor's continued fertility.

In vitro embryo production generally consists of the three-step process of oocyte maturation, oocyte fertilization, and embryo culture. Following recovery, cumulus-oocyte complexes at the germinal vesicle stage of meiosis are placed into medium designed to stimulate maturation to the second-metaphase stage of meiosis. In vitro oocyte maturation generally occurs over a 24-hour period, after which the mature oocytes are exposed to capacitated sperm for an additional 12 to 24 hours. Sperm capacitation can be induced by exposure to various chemical or biologic factors. Following fertilization, presumptive zygotes are generally placed into culture for an additional 5 to 7 days, during which time the developing embryos cleave to stages that can be cryopreserved or transferred to the uterine lumen (compact morulae and blastocysts).

In vitro embryo production systems have been recently developed in red deer.[9–11] Laparoscopically recovered red deer oocyte-cumulus complexes can be matured and fertilized in the laboratory, with the resulting embryos cultured to advanced stages. In vitro maturation procedures have generally mimicked standard bovine or ovine protocols using either purified hormones or biologic fluids as the in vitro gonadotropin source. In vitro sperm capacitation has been induced in red deer sperm with either purified heparin or estrus sheep serum. The culture of in vitro derived presumptive zygotes has resulted in variable results and relatively few embryos developing to advanced embryonic stages. The establishment of embryo transfer–based pregnancies involving red deer IVP embryos has apparently been restricted to the transfer of early stage embryos.[10] The transfer of later stage (morulae and blastocysts) in vitro–derived red deer embryos has thus far not resulted in the establishment of viable pregnancies. As red deer hinds respond at relatively high rates to standard superovulation protocols and with the recent evolution of nonsurgical embryo recovery and transfer systems in this species, the use of developing IVP systems may be restricted to use with either juvenile hinds or in the treatment of infertility.

Because of this inability to successfully superovulate wapiti on a repeatable basis, a significant effort has been put forth toward the development of methods for the in vitro production of wapiti embryos. As even nonstimulated ovaries contain accessible populations of small diameter follicles, immature oocytes derived from these follicles can be matured and fertilized in the laboratory in order to produce viable embryos for transfer. Oocytes can be collected from mature wapiti cows by transvaginal aspiration on a weekly basis without adverse effect on subsequent donor fertility. Early attempts at IVP in wapiti demonstrated that, as in red deer studies, embryos could be successfully produced and that these embryos would result in pregnancies if transferred at very early cleavage stages.[12] In these early studies, IVP wapiti embryos cultured to more advanced embryonic stages were not able to survive cryopreservation nor produce viable pregnancies if transferred. More recently, however, embryo culture systems have been evolved that produce embryos of advanced embryonic stages that are able to produce high pregnancy rates upon transfer and survive cryopreservation. These methods have now been advanced by our group to allow for the production of large numbers of wapiti calves derived from the in vitro fertilization of oocytes recovered from juvenile wapiti donor calves (3–4 months of age). The production of wapiti embryos and resulting embryo transfer calves from oocytes laparoscopically recovered from juvenile wapiti donors, allows for greatly accelerated genetic progress in a species normally possessing long generation intervals. The transfer of embryos derived from oocytes recovered from 3- to 4-month-old heifer calves has resulted in pregnancy rates following surgical transfer to red deer/wapiti hybrid recipients of 65% for fresh embryos and 61% for cryopreserved embryos (Table 136-4). The current state of IVP technology in farmed wapiti allows oocytes recovered laparoscopically from juvenile calves or transvaginally from mature cows to be fertilized and cultured in the laboratory so to produce blastocyst stage embryos that survive cryopreservation and transfer at high rates. No malformations, abnormal birth weights, or lengthened gestations have been observed to date from calves produced from the transfer of IVP wapiti embryos.

Table **136-4**

Calving Rates Following Surgical Transfer of Fresh or Cryopreserved in Vitro Produced Wapiti Embryos*

	Fresh Embryos	Cryopreserved Embryos
Total number of embryos transferred†	55	55
Total number of recipients calving	36	33

*Embryos derived from oocytes recovered from juvenile wapiti donors (3–4 months of age).
†Grade 1 or 2 IVP wapiti blastocysts.

Embryo Cryopreservation

Cervid embryos are exceptionally robust and amenable to manipulation including cryopreservation. To date, the vast majority of cervid embryos that have been cryopreserved commercially have and continue to be frozen utilizing glycerol as the cryoprotectant. Standard bovine protocols making use of 1.5 M glycerol cryoprotectant solutions, freezing rates of 0.3 to 0.4°C per minute, and a three-step dilution process have been typically utilized. Although the majority of cervid embryos have been

cryopreserved at the compact morula and blastocyst stages, red deer embryos cryopreserved as early as the 8-cell stage have resulted in the birth of live fawns following transfer. As in other domestic ruminant species, the transfer of cervid embryos cryopreserved in glycerol has typically averaged 55% to 65% pregnancy rates (Table 136-5). A limited number of transfers involving blastocysts have additionally demonstrated that wapiti embryos may be successfully cryopreserved using ethylene glycol as the cryoprotectant for freezing and direct dilution during the thawing process.

Embryo Transfer

Embryos created and recovered by whatever means must be transferred to suitable recipients for viable offspring to be produced through embryo transfer. The overall pregnancy rate resulting from the transfer of cervid embryos is dependent upon a complex interaction of factors, including the health and viability of the transferred embryo, the synchrony established between the age and development stage of the embryo and the recipient's uterine environment, the health and reproductive condition of the selected recipient, and the clinical expertise and skill of the embryo transfer team. Red deer hinds are generally selected as recipients for red deer embryos, and red deer/wapiti hybrids have been primarily utilized as recipients for wapiti embryos. The use of pure red deer recipients for wapiti embryos generally results in increased dystocia rates due to the larger size of wapiti calves at birth in comparison to red deer fawns. Recipient hinds are synchronized using methods identical to those used for artificial insemination. In brief, a single CIDR-G in red deer and either two CIDR-G or a single CIDR-B for red deer/wapiti hybrids is inserted within each recipient on day 0. The CIDR is removed on day 12 in red deer and on day 14 in red deer/wapiti hybrids, with 200 IU of eCG administered intramuscularly. Recipients are synchronized to come into heat ~24 hours behind that of donor hinds or cows (48 hours following follicular aspiration for IVP embryos). The transfer of red deer or wapiti embryos to red deer or red deer/wapiti hybrid recipients has been, until very recently, performed surgically or laparoscopically. Laparoscopic transfer methods in cervids has evolved to a procedure more appropriately termed a laparoscopically guided minilaparotomy. By these methods, a laparoscope is inserted into each recipient's abdomen while it is under general anesthetic (recipient hinds are removed from water and feed for at least 12 hours prior to surgery) in order to visualize both the recipient's ovaries (so to confirm their reproductive status—presence and condition of corpus luteum) and ipsilateral uterine horn to facilitate exteriorization. Once the recipient's reproductive status is confirmed, a small midventral incision (3–4 cm) is then made and the tip of the isolateral uterine horn is exteriorized with atraumatic gasping forceps. A blunted 19-gauge needle is passed through the uterine wall several centimeters caudal to the uterotubal junction, and the embryo is transferred to the recipient's uterine lumen using either a tom-cat or IVF catheter. Pregnancy rates produced by our group between 2000 and 2002 averaged above 55% across several hundred surgical procedures involving the transfer of single cryopreserved embryos to synchronized red deer recipients (see Table 136-5). In contrast, the transfer of single fresh red deer embryo to synchronized red deer recipients generally results in pregnancy rates above 70%.

Our working group has also pioneered and tested the transcervical transfer of cryopreserved red deer or wapiti embryos to nonanesthetized standing pure red deer or hybrid recipients (Fig. 136-1). Recipient hinds

Table **136-5**

Comparison of Fawning Rates Following Either Surgical or Transcervical Transfer of Cryopreserved Red Deer Embryos

Feature	Surgical Transfer	Transcervical Transfer
Total number of embryos transferred*	484	75
Total number of recipients	273	48
Fawning		
Fawning rate (%)	56	64
Calving rate (%)	65	61

*Cryopreserved grade 1 morulae or blastocysts.

Fig. 136-1 Embryo collection using a Foley catheter in a wapiti hind. (From Haigh JC, Hudson RJ: *Farming wapiti and red deer*, St Louis: Elsevier, 1993.)

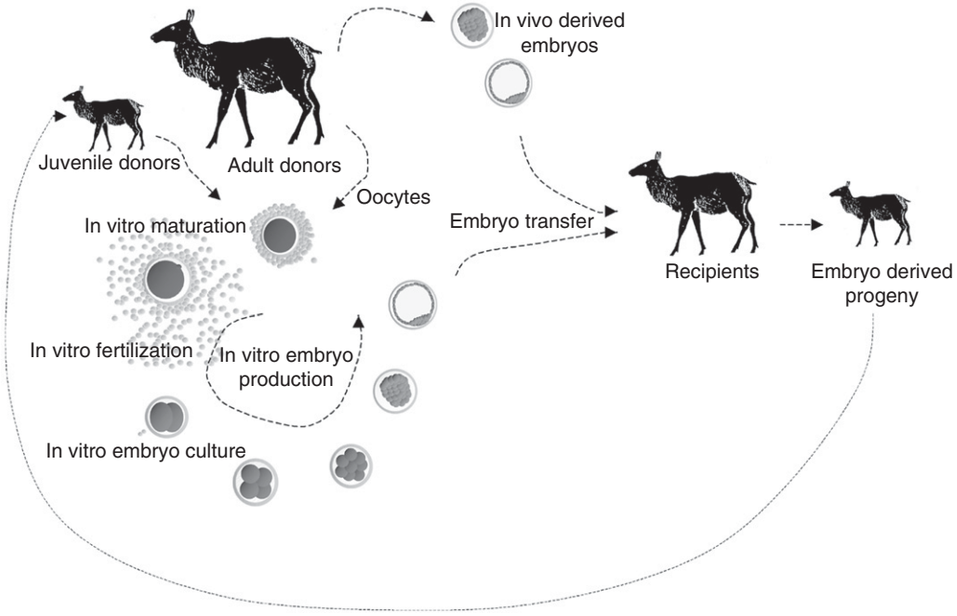

Fig. 136-2 Application of embryo biotechnologies in cervid breeding.

synchronized as for surgical transfer are held stationary in a hydraulic crush. An epidural anesthetic is applied and the rectal contents are removed. The ovaries and uterus are rectally palpated to determine the recipient's reproductive status and suitability. A small-gauge blunted metal cannula is passed through the recipient's cervix and into lumen of the isolateral uterine horn. The embryo loaded within a long polyethylene transfer catheter is then injected into the uterine lumen using a minimum volume of fluid. Transcervical transfer of red deer embryos to recipients has resulted in pregnancy rates of ~60% and appears comparable to that following surgical transfer (see Table 136-5). Transcervical embryo transfer benefits both recipient animals and breeders because the procedure is much less stressful and time-consuming for both parties.

FUTURE APPLICATIONS IN CERVID BREEDING

The application of embryo-based biotechnologies to cervids will evolve in response to economic and production forces driving the cervid breeding industries. One such economic force at play in North America is the relative high value of wapiti cows in comparison to either pure red deer or hybrid hinds. As wapiti cows are valued at several times that of hybrid animals, wapiti calf production systems taking advantage of the genetic compatibility between wapiti and red deer through the transfer of pure wapiti embryos to low cost hybrid hinds will continue to develop. Embryo biotechnologies will also contribute by minimizing the cost of genetic transport and risk of disease transmission during domestic and international genetic exchange. The importance of limiting the risk of pathogen transmission in farmed wapiti and red deer during genetic exchange continues to increase owing to the emergence of devastating diseases such as chronic wasting disease and tuberculosis. Embryo biotechnologies will also function to advance genetic improvement in cervids by the shorting of generation intervals through use of juvenile oocyte donors (Fig. 136-2). The innovative nature of the cervid breeding industries, combined with increasing demand for genetic improvement and exchange, as well as the need for pathogen elimination, shall continue to increase the clinical utilization of embryo biotechnologies in this species.

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CHAPTER 137

Reproductive Patterns in Female Bison (*Bison bison* sp.)

KAREN L. GOODROWE, GABRIELA F. MASTROMONACO, and LEANNE S. OTHEN

REPRODUCTIVE CHARACTERISTICS

The female bison is seasonally polyestrous, with estrous cyclicity usually beginning in late July or August and conception generally occurring at the first or second estrus and a reported 95% of cows mating only once.[1–4] The length of the bison estrous cycle has been reported to range from 16 to 31 days, with an average length of 20.8, 21.5, and 20.8 days based on behavioral observations, and urine and fecal progestin profiles, respectively.[2,3] Although estrogens can be measured in cycling female bison, the profiles do not provide physiologic indicators of behavioral estrous or ovarian activity.[5] Based on endocrine evaluations of luteal activity, behavioral signs of estrus, and recorded births, females can continue to cycle through December or January.[3,6] Demonstrations of behavioral estrus include homosexual mounting, standing for mounting, flehmen, and tail flagging, and these behaviors coincide with nadir progestin values in cycling animals.[3,5,7] The behavioral ecology of male and female bison, with particular emphasis on reproductive behaviors, has been well described by Berger and Cunningham.[4]

To establish population demographics and reproductive characteristics in bison, historical records from the Toronto Zoo wood bison herd were analyzed from 1978 to 2000.[6] Of the 145 bison calves born during this time, all were singletons. A total of 14 calves (9.6%; 8 males, 4 females, and 2 unknown) either were stillborn or died on the day of birth. Eleven calves (7.6%; 3 males, 8 females) died within 30 days post partum. Parturition most frequently occurred in May (Fig. 137-1, 55.4%). This is consistent with data from free-ranging plains bison; however, animals living at higher latitudes or in areas with more harsh winters have demonstrated a trend to produce calves later in the spring.[4] From the Toronto Zoo records, calves born from August to October had a greater mortality rate (37.5%), compared to those born between April and July (15.5%).[6] The sex ratio of offspring was approximately equal: 51.0% females and 47.6% males. The youngest and oldest age at which a female produced offspring was 2 and 17 years, respectively. The mean age of females producing offspring (from females of known age, $n = 102$) was 6.01 years. On average, both bulls and cows lived between 14 and 17 years.

Calf production in free-ranging plains bison has been reported to be greatest in females aged 5 to 13, with those aged 10 to 13 years being the most fecund, and more than 80% of pregnancies occurring in animals 4 years of age or older.[4,8] Pregnancy rates have been reported in lactating cows and are attributed to ovulation failure.[8] Although rare, yearlings have been reported to conceive.[1,4] Pregnancy rates of 70% have been reported for free-ranging bison.[1] Observations of 16 plains bison births have shown that parturition is rapid, with birth occurring an average of 68 minutes after the amnion was first visible and 13 minutes after the forehooves were first observed.[9] Calves were precocial and stood and nursed 10.8 and 32.2 minutes, respectively, after birth. In open

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CHAPTER 137

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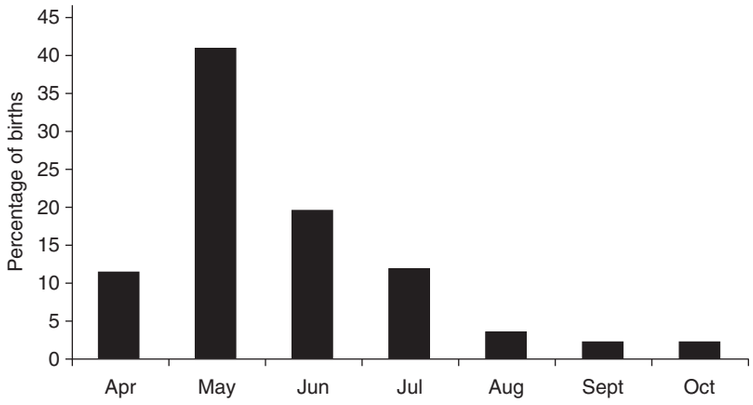

Fig. 137-1 Frequency distribution of births in a captive herd of wood bison at the Toronto Zoo over a 22-year period (n = 145).

habitat, cows tended to remain in groups, whereas in wooded areas, cows tended to give birth in isolation.[9]

PREGNANCY

There are no previous reports documenting hormonal patterns during the entire period of gestation in bison. To establish longitudinal steroid profiles for the wood bison during pregnancy, fecal and urine sample collection was performed at least five times weekly over three reproductive seasons (August until birth) from a captive herd of wood bison at the Toronto Zoo. The actual number of samples for each individual varied, but samples were taken at least once each week. Samples were analyzed for steroid hormone concentrations by enzyme immunoassay (EIA) using previously described methods.[3,5,7] Urine was analyzed for pregnanediol-glucuronide (PdG), estrone conjugates (EC), and cortisol, and feces were analyzed for progestins (P) and EC. Steroid hormones were extracted from feces according to previously described methods and stored frozen until analysis.[5,7] Gestation length was determined from observed mating or last observed estrus until the birth of a calf.

Hormonal patterns were monitored in 11 pregnancies over the 3-year period. One calf from year 1, one calf from year 2, and two calves from year 3 were stillborn, and their respective dams died due to complications during or after calving. Two of the stillbirths were female and two were male. The mean gestation length for the 11 pregnancies was 265.3 ± 2.5 days. This differs from reports of primiparous and multiparous free-ranging bison (287.9 versus 279.9, respectively). Berger and Cunningham have reported adjustment of gestation length in females, with those in good body condition experiencing an ~6 day shortening of gestation.[4] No difference was detected between pregnancies producing live offspring (267.9 ± 1.0 days) and stillbirths (263.2 ± 6.8 days) or between pregnancies carrying male and female calves in the Toronto Zoo wood bison herd. Physical attributes indicative of impending parturition included: vulval swelling 2 to 3 weeks prepartum, mucus secretion approximately 2 weeks prepartum, and udder development. On the day of or just preceding parturition, increased restlessness, separation from the herd, passing of the mucous plug, and contractions were observed.[5]

Live Births

Progestins

Composite weekly PdG and P profiles from the viable pregnancies are shown in Figure 137-2. Urinary PdG concentrations were unchanged from week –37 until week –14, then increased markedly from week –13 to week –7. After week –7, PdG levels declined gradually to 749.0 ± 148.1 ng/mg creatinine at week –5, followed by a more dramatic decrease at week –4, which continued through week 0. Fecal P levels demonstrated a pattern similar to the PdG profile (see Fig. 138-2). Mean P concentrations did not differ from luteal phase values between week –37 and week –12. A significant elevation in P was detected at week –10 (920.56 ± 124.6 ng/g feces). Fecal P values then continued to rise to a peak at week –4, after which time levels markedly declined through week –2 and continued to fall through parturition. Fecal P concentrations had not reached baseline by week 3 post partum.

Estrone Conjugates

A doubling in mean urinary EC from mean nonpregnancy levels of 1.0 ± 0.1 ng/mg Cr to 2.2 ng/mg Cr was detected between weeks –35 to –33 (Fig. 137-3). Urinary EC then remained statistically unchanged, but increased gradually, from week –31 to week –5, then increased rapidly between week –4 to a peak at week –1 (range: 475.2–2272.7 ng/mg Cr). A precipitous decline in urinary EC was observed between week –1 to week 0 and decreased even further by week 3 post partum. Fecal EC concentrations increased markedly from mean nonpregnancy levels (25.4 ± 5.7 ng/g feces) between weeks –30 to –26 (see Fig. 137-3), similar to the pattern observed for urinary EC. Fecal EC concentrations remained statistically unchanged from week –30 to week –5, but also showed a gradual increase over time. A significant elevation in fecal EC concentrations was detected at week –4 (295.5 ± 29.9 ng/g feces) which increased in all females to a peak during week –1. Fecal EC concentrations declined in the week prior to parturition and continued to fall through week 3.

Corticosteroids

Mean urinary corticosteroid levels from the seven viable pregnancies remained consistent throughout gestation,

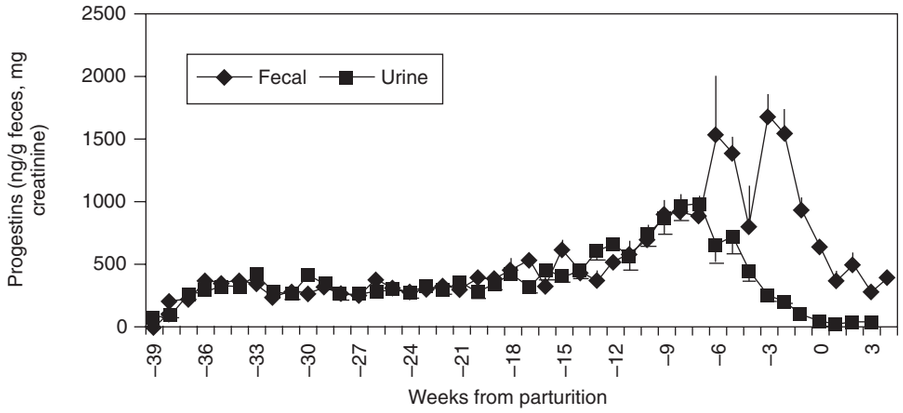

Fig. 137-2 Mean weekly fecal progestin (◆) and urinary pregnanediol-glucuronide (■) concentrations during gestation in seven wood bison at the Toronto Zoo. Data are aligned to the week of parturition (week 0).

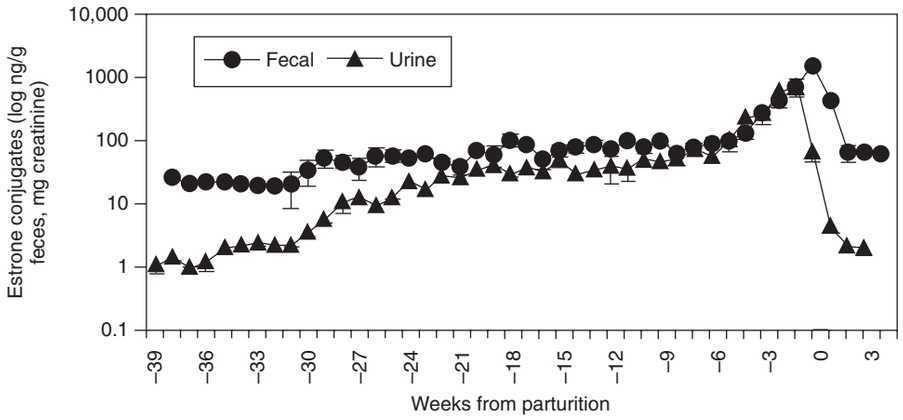

Fig. 137-3 Mean weekly log of fecal (●) and urinary (▲) estrone conjugate concentrations during gestation in seven wood bison at the Toronto Zoo. Data are aligned to the week of parturition (week 0).

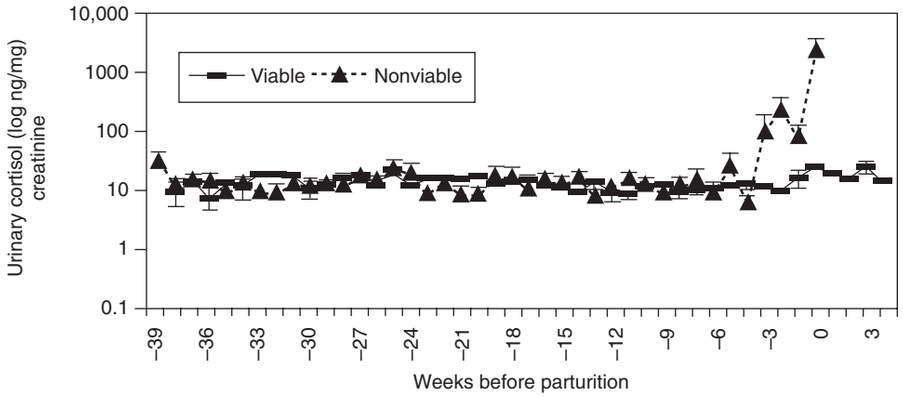

Fig. 137-4 Mean weekly log of urinary corticosteroids during gestation from seven viable (—▬—) and four nonviable wood bison pregnancies (calves stillborn; ▲) at the Toronto Zoo. Data are aligned to the week of parturition (week 0).

with a mean of 14.3 ± 0.5 ng/mg Cr (range 1.8–23.4 ng/mg Cr). During the week prior to parturition, urinary corticosteroids demonstrated a marked elevation to 28.1 ± 6.1 ng/mg Cr (Fig. 137-4). Corticosteroids were not measurable in feces.

Stillbirths

Similar endocrine patterns for PdG, P, urinary EC, and fecal EC were observed among the four females that produced stillbirths, and mimicked those of viable pregnancies. However, significant elevations in corticosteroids (10- to 100-fold greater than values observed in viable pregnancies) were observed in weeks –3 through 0 (see Fig. 137-4). Collectively, this information indicates that both the peak in estrogens and corticosteroids can be used as an indicator of impending parturition, and that corticosteroids may provide insight into the well-being of the fetus up to 14 days prior to parturition.

Table **137-1**

Pregnancy Diagnosis Based on Fecal Estrone Conjugate Analysis*

Estrone Conjugate Levels	Prediction	Outcome
>5 ng/g feces	18 pregnant†	17 pregnant
4.0–4.9 ng/g feces	6 possibly pregnant‡	5 pregnant
<4 ng/g feces	19 not pregnant	21 not pregnant

*Analysis based on 43 female wood bison at approximately week –27 of gestation.
†One false positive.
‡One animal was not pregnant.

Table **137-2**

Pregnancy Diagnosis Based on Fecal Estrone Conjugate Analysis*

Estrone Conjugate Levels	Prediction	Outcome
>25 ng/g feces	29 pregnant	29 pregnant
20–24 ng/g feces	13 possibly pregnant	3 pregnant,† 10 not pregnant
<20 ng/g feces	35 not pregnant	33 not pregnant, 1 abortion,‡ 1 pregnant

*Analysis based on 76 female wood bison at approximately week –14 of gestation.
†The three pregnant animals gave birth later in the year (from July through October), and the 10 animals not pregnant were yearlings.
‡The spontaneous abortion occurred in March.

Pregnancy Diagnosis

Based on the preceding profiles, fecal samples were collected in a single breeding season from 43 wood bison females of the Great Slave Lake herd, Northwest Territories, Canada, to test the feasibility of pregnancy diagnosis based on single samples. The first occasion for sample collection was in November, which retrospectively was (on average) week –27 of gestation. Using the EC assay, fecal samples were analyzed and diagnosis of reproductive status was made using the following criteria: over 5 ng/g feces = pregnant; 4.0 to 4.9 ng/g feces = possibly pregnant; and below 4 ng/g feces = not pregnant (Table 137-1). Over a 2-year period, 77 fecal samples were collected from the same herd of animals in February (at ~week –14 of gestation). Samples were analyzed for fecal EC and evaluation of reproductive status was conducted using the these criteria: over 25.0 ng/g feces = pregnant; 20.0 to 24.0 ng/g feces = possibly pregnant and below 20 ng/g feces = not pregnant (Table 137-2). In animals deemed to be pregnant at week –27, there was a 94.45% accuracy of positive pregnancy detection, but for animals predicted to be pregnant at week –14, there was 100% accuracy. Overall, pregnancy diagnosis performed from fecal samples collected during midgestation was a reliably high predictor of pregnant animals.

Alternatives to fecal hormone analysis for bison pregnancy detection have been described. Kirkpatrick and associates used urine samples collected over a 30-day period to diagnose pregnancy in plains bison.[10] Diagnosis was made based on noncyclic patterns, which demonstrated continually increasing PdG concentrations (>200 ng/mg Cr). The earliest detection of pregnancy in wood bison was between weeks –30 and –26 of gestation using fecal ECs. Urinary ECs also could be used as an indicator between weeks –35 and –33. This is earlier than reported by Kirkpatrick and associates, who were unable to determine pregnancy with urinary EC until 3 to 4 months (weeks –26 to –21) of gestation.[11] The tendency for both urinary and fecal progestin concentrations to remain comparable to peak luteal phase levels for the majority of gestation renders progestins to be less reliable indicators of early pregnancy. Detection of pregnancy in plains bison using total fecal estrogens (>1.09 ng/g feces) and PdG (>57.7 ng/mg Cr) has been found to be 100% accurate after 90 days of gestation, but urinary estrone conjugates (>10 ng/mg Cr) were 89% accurate determining pregnancy in the second month of gestation.[10] Diagnosis of pregnancy in wood bison sampled from November to January has been assessed by radioimmunoassay (RIA) for pregnancy-specific protein B (97% reliable) and by rectal palpation (89% accuracy).[12]

From a herd husbandry perspective, improved understanding of reproductive processes in bison can contribute to more effective management practices. In captive populations, longitudinal monitoring of pregnancy can indicate fetal health, determine abnormalities in gestation, and estimate the date of parturition, enabling appropriate animal care preparations to be made and allowing early problem recognition. Interestingly, the endocrine patterns of pregnancy during gestation in bison do not mimic those of cattle, but rather demonstrate characteristics similar to both sheep and cows.[13] As in sheep, fecal and urinary progestin levels were consistent with peak luteal phase values for the first trimester, became elevated during the second trimester, and declined 3 to 5 weeks before parturition, respectively; whereas estrone conjugate concentrations in feces and urine increased markedly 4 weeks prior to parturition, similar to domestic cattle. This unique hormonal profile suggests that interspecies embryo transfer between bison and cattle would be ineffective, as has been demonstrated for the domestic and Dall's sheep, and further stresses the need to establish species-specific databases, rather than relying on a presumed model.[14]

ASSISTED REPRODUCTIVE TECHNOLOGIES

There is a paucity of information concerning assisted reproductive technologies in bison. Despite reproductive similarities to cattle, reliable and effective methods for estrous synchronization, superovulation, embryo transfer, and artificial insemination have not yet been developed. Rectal ultrasonography can be used to assess ovarian activity in bison.[3,5] This is most successful when

the animals have been habituated to human presence and restraint facilities. Bison ovaries and corpus luteum (CL) average 26 × 18 mm and 22 × 19 mm in size, respectively, and follicles range in size from 7 × 7mm to 10 × 14 mm.[3] In a single report of successful embryo transfer, estrous cycles were synchronized in both donor and recipient cows using Syncromate B implants (SB) with combinations of estradiol valerate (E_2V) and prostaglandin.[15] Superovulation of donor cows was attempted with either pregnant mare serum gonadotropin (PMSG) or follicle-stimulating hormone (FSH). Recipients received either gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) to synchronize ovulation and were artificially inseminated with fresh semen. Only moderate ovarian responses were obtained from donor females, with 28 of 33 attempts to superovulate 16 females resulting in sufficient responses to conduct embryo collections. A total of 20 viable embryos (0.71 embryos/collection attempt), 11 degenerate embryos, and 63 unfertilized ova (2.25 UFO/collection) were collected. Other attempts at synchronization and superovulation have used permutations of the preceding protocols, but with limited success.[3,7,16] Reliability of E_2V in combination with SMB implants from both an ovarian and endocrine response perspective led one group of investigators to choose prostaglandin F over E_2V as a vehicle for estrous synchronization.[7] There are no reports of IVM with bison oocytes, and only two reports of in vitro fertilization (IVF) to produce hybrid embryos using bison sperm to inseminate cattle oocytes.[17,18] In both cases, standard cattle IVF protocols were used for in vitro maturation and IVF. Fertilization rates were moderate, but embryonic development rates were low, indicating that extensive work is necessary to define optimal sperm preparation/processing and culture conditions.

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CHAPTER 138

Reproductive Management of Bison

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REPRODUCTIVE BIOLOGY

Bison in North America are seasonal breeders. Using data collected from several different locales in the Northern Hemisphere, Berger and Cunningham reported that in any given year the earliest calves are seen in the first week of April, and that 25% of births had occurred by April 22. The median birth, over a 5-year period, always fell between May 2 and 8, and 80% of all births had taken place within 55 days of the first one recorded.[1] In Northern Alberta the onset of calving often occurs about a week earlier than this. Some later calves may be born in November or even December. A management scheme that describes the events in a bison ranch year is presented in Table 138-1.

Actual mechanisms of control of seasonality have not been studied, and the possibility that the high degree of synchrony is driven by availability of suitable nutrition does exist. Anecdotal evidence from bison ranchers implies that no calvings are seen in the months of January, February, and the first half of March, although such calvings have occasionally been reported.[2]

On average, bison bulls reached puberty at 16.5 months and should be capable of passing a breeding soundness evaluation by 24 months of age.[3]

Bison bulls demonstrate a moderate degree of seasonality that is evident in changes of fecal testosterone, testicular histology, and to a limited degree in semen quality.[4]

Rutley and Rajamahendran,[5] in a study of nine plains bison and five wood bison, found that females are seasonally polyestrous and go through a period of about 3 months when there is no evidence of ovarian activity. Using serum and fecal progestin metabolites as indicators of ovarian activity they found that there was a significant difference in concentrations between periods of anestrus and pregnancy, and that open cows entered a period of anestrus in spring. However, they found considerable overlap between fecal progestins of pregnant animals and those not bred, but exhibiting periodic estrus, and even some overlap between nonbred animals and those in anestrus. A similar study involving behavioral observations and the evaluation of fecal progesterone and urinary pregnanediol glucuronide showed that cyclic activity began in early August and ceased in early December.[6]

Social integration among bison cows can create problems that are not seen in beef herds. When different groups of cows are mixed a change of dominance position can cause delays in the onset of estrus. The animals may take a considerable time to integrate.

PRODUCTIVITY

The calving rate and number of calves per adult cow bison vary widely according to management system, particularly nutritional status.

In some free-ranging herds the productivity is much less than in managed operations. For instance, five parameters of reproductive biology of the free-ranging herds in Yellowstone National Park were examined by Kirkpatrick and associates.[7] They found that lactating cows younger than 5 years of age did not conceive, a fact caused by ovulation failure, and that range condition had an effect upon conceptions in cows 4 years old or younger. The pregnancy rates reported for cows of this age would not be acceptable in commercial herds.

However, calving rates for bison in the National Bison Range, Montana, were between 78% and 100%.[8] Kirkpatrick and associates[7] considered that the three factors of environmental conditions, age, and lactational state influence the overall reproductive performance of the bison in their study, and it is likely that these factors play a role in any mammalian population.

Successful bison managers control parasite loads in their herds either through good pasture management, or the use of anthelmintics at strategic points in the calendar.[9] Of particular concern are the stomach worms of the family Ostertaginae, which have been shown to cause death in both winter and spring, mainly through type II ostertagiasis, or in summer by causing debility and reduced productivity, including reproductive failure.[10]

Good pasture management is essential for the success of breeding programs in bison operations. It is common practice to "flush" bison before the onset of the rut, and ensure that cows enter the breeding season in good body condition.[7] At this time most ranched animals are also feeding calves, and on well-managed properties an objective is to achieve as close as possible to a 100% conception rate, even in lactating animals.

Good nutritional management is also essential in the spring months. Dystocia is reported by bison ranchers, and there is little doubt that overfat cows have an increased risk of dystocia, although this has not been critically tested.[9]

Table **138-1**

Reproductive Management Calendar—Bison

Month	Calves	Cows	Bulls	Nutrition
Jan	Late weaning	Late pregnancy check	Very late breeding	Begin to decrease nutrition
Feb				Aim for BCS of 2.5 at calving
Mar			Semen testing of bulls	
Apr	Calving starts		Semen testing	
May	90–95% calving		Semen testing	Increase plane for cows as they calve
June	Some may go as late as Dec (see text)			Grass ideal
July			Breeding	Aim for BCS of 4.0 for cows through to December
Aug			Breeding	
Sept			Breeding	
Oct	Weaning	At weaning vaccinate and deworm	Breeding (may remove at this time)	
Nov	Weaning	Pregnancy check	Some continue with breeding	
Dec	Late weaning	Pregnancy check		Decrease plane

BCS, body condition score.

Table **138-2**

Body Condition Scoring—Bison

Score	Fat cover	Ribs	Spine	Flank	Inguinal fill	Thurl
1	None	All visible	Very sharp	Empty	Very sharp	Sunken
2	Minimal; at necropsy some fat visible in gastrointestinal tract	All visible	Very sharp	Empty	Sharp	Flat
3	<1 mm backfat	7+ visible (depends on hair coat)	Just visible			Slightly rounded
4	2–12 mm backfat (classifies as A1 on slaughter)	Less than 4 visible	Not visible	Thickened	Rounded	Round
5	>12 mm backfat (usually >20 mm)	1 or 2 may be visible, but covered	Not visible	Full	Deep	Lumpy

BODY CONDITION SCORING

No formal data have been reported for the use of body condition score (BCS) in bison, and the heavy winter coat can confound accurate field examination. However, a field system of scoring can be used (Table 138-2). Palpation is not an option. Using this system a target BCS of 2.5 (on a scale of 5) can be used for animals entering the breeding season.

To understand BCS in bison it must be realized that they deposit fat in a manner more similar to dairy animals than beef cattle. Fat is deposited first (and predominantly) in the interstitial spaces and in the abdominal cavity. With increasing obesity a variable layer of subcutaneous fat, and marbling, is deposited. The subcutaneous layer is not uniform but occurs most commonly over back and loin, dorsal neck, near the tail-head, and in the groin and flank. Back fat measurements of 30 mm are occasionally seen at slaughter. Marbling within muscles is seen only in obese animals.

Animals in a negative energy situation utilize fat reserves. Subcutaneous fat pads (such as those near the tail-head) disappear first, which leads to clearer muscle definition as the body loses condition. This is especially noticeable on the hindquarters in summer. As interstitial fat is removed muscles appear to subside, dorsal spinous processes seem to protrude more, the neck narrows, and ribs and pelvic structures become more visible. In very thin animals all fat has been metabolized and muscle mass is used as an energy source (see Table 138-2 and Figs. 138-1 to 138-4).

FERTILITY TESTING

Bison bulls are often tested for fertility in May before the breeding season, although many farmers do not test bulls more than once in the bull's lifetime. Testing is also carried out at other times of year, for instance, before auction sales. Bison bulls can produce semen year-round

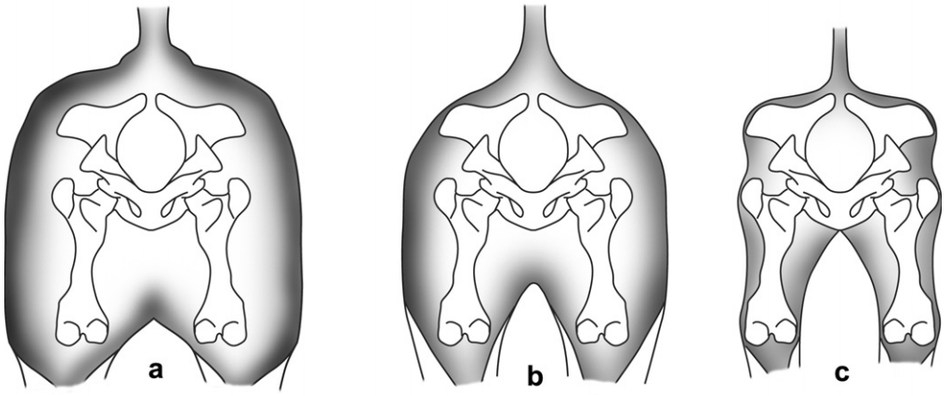

Fig. 138-1 Representation of three body condition scores (BCS) showing view from the rear. Roundness of the hip and buttock region and the angle of the inguinal region indicate gradation from obese (**a,** BCS 5) through "good working condition" (**b,** BCS 3) to extreme emaciation (**c,** BCS 1).

Fig. 138-2 Bison with a BCS of 1. This animal is extremely emaciated. It died within 1 week of the picture being taken.

Fig. 138-4 Bison with a BCS of 4.5 to 5.0. The most noticeable feature is the presence of large irregular masses of fat around the tail-head.

Fig. 138-3 Two bison with BCS of 3.0.

and examinations of seasonal fluctuation in ejaculate quality have shown that fluctuations do occur, which provides a caveat for those conducting test in late winter or early spring.[3] These tests are limited, for the most part, to semen collection by electroejaculation (e-e) followed by its evaluation. Libido testing has not been reported, and it is generally considered too dangerous to carry out scrotal circumference measurements, although these can be done in suitably designed chutes.[11] Bison bulls can kick backward with great force and standing directly behind them is not recommended.

In vitro fertilization with either fresh, or frozen-thawed, semen has been used in the production of bison × domestic cattle hybrid embryos and could be employed as a means of testing semen fertility, but is not practical for the bison rancher.[12]

The only objective report so far published has been the study carried out for 5 consecutive years on a total of 234 bison bulls in the 28- to 30-month-old age group at Custer State Park.[11] The 1992 Society for Theriogenology guidelines for beef cattle semen evaluation and

reproductive tract examination were used. The authors concluded that scrotal circumference (SC) was significantly correlated with body weight, percentage of normal spermatozoa, percentage of primary spermatozoal defects, and percentage of motile spermatozoa. SC was positively associated with increased odds of semen collection, satisfactory motility, satisfactory morphology, and simultaneous satisfactory motility and morphology. Receiver-operator characteristic curve analysis selected 29 cm as the optimal SC cutoff most predictive of simultaneous satisfactory spermatozoal motility and morphology. The authors further concluded that SC is a good indicator of adequate spermatozoal motility and structure in bison.

The authors also pointed out that the study was limited by the fact that all samples were collected in early October, which is essentially after the end of the breeding season, taking no account for possible seasonal variations. They also pointed out that all the animals came from one location.[11]

To accomplish a quality examination, the bull must be restrained in a squeeze that will hold him securely so he will not injure himself or the evaluator. The squeeze must also allow unrestricted access to the rear as well as the lower side of the animal. Many bison bulls have a tendency to squat when held in a chute, and this often means considerable delays in the procedure. Some ranchers have a tendency to apply electric prods randomly over the animal's body in an attempt to make him stand square. This is seldom justified, and usually means that the animal simply becomes obstreperous. If a prod is to be used, its judicious application, with a couple of very brief stimuli to the coronet of the hind foot, will usually make him stand, and a couple of applications, if needed, will often train a bull. A better option is to use a hydraulic chute designed to prevent squatting.

An alternative approach has been employed in a zoo setting, where a small exhibit herd is maintained. The animals are habituated to human presence, and to the chute by keeping it open and allowing them to wander in and out and having the chute associated with the feeding area. This approach generally results in calmer animals when they are handled in the chute for any type of processing.[13]

When semen evaluation is conducted, evacuation of feces from the rectum and palpation of the pelvic accessory sex glands can be carried out from one side. During this examination ampullary massage can be conducted, and this will often aid the entire collection process. After carrying out a rectal examination, an operator may move a hand down to the scrotum in a single continuous move, maintaining contact with the perineal skin, and palpate the scrotum for abnormalities, but caution must be exercised during this procedure. The testicles can be checked for consistency and their sizes can be compared. Any abnormalities, including monorchidism, are noted.

A variety of units are used for electroejaculation, which can be considered part art and part science; thus, some of the variations have to do with operator confidence and experience as much as anything. During the ejaculation process, the penis is visually inspected for damage, defects, or the inability to extend. Handling of semen should follow established techniques used for the domestic bull and at certain times of year may involve the use of a warm-water sleeve outside the collection tube, or a preheated Styrofoam cup. Often the owner needs the information quickly because he is sorting the bulls based on the results of the reproductive evaluation.

Most bison bull semen examination involves the same measurements as used for domestic cattle. Gross motility is scaled either from 1 to 3 or 1 to 5, the highest rating being akin to a mass of swirling clouds, the lowest barely moving.

Individual motility is detected under higher magnification. The minimum requirement for beef animals is 30%. Some veterinarians use this standard for bison as well. It is possible that this standard should be higher for bison. In an evaluation form designed for one client, the minimum accepted standard for individual motility is 50%.[14] However, there are no objective data that confirm or refute this figure. It may be that bulls meeting 30% may be able to breed successfully.

Unlike the beef industry, bison bulls at 18 to 24 months of age can easily be moved into a feeder program rather than being maintained as breeding stock. Some producers prefer to have only the superior bulls used for breeding and therefore request the higher minimum standards.

Morphologic criteria are the same for bison bulls as for domestic cattle. The minimum standard accepted for use of a bull in a breeding program is generally taken as 70% normal cells. Nigrosin-eosin is a useful stain, and a Feulgen stain has been used for the examination of nuclear material.[3,4]

Using Society for Theriogenology beef standards, it has been demonstrated that young bulls do not always pass the first time, as they may not have reached sexual maturity. The commercial bison industry semen tests, and possibly rejects, many developmentally incomplete bulls between 19 and 21 months of age. Studies by Helbig and associates have shown that a failed test at 21 months may be too soon to discard a bull and that those that fail semen tests at this age may be worth testing again at 24 months.[3]

Table 138-3 shows results of semen evaluation with the following minimum requirements: individual motility of 50%, morphology 70% normal.[10,14] No statistical analyses were conducted, but it appears that the percentage of samples rated as satisfactory increases around the age of 2 years.[14]

Keen and associates[11] in their study of the 28- to 30-month-old bulls at Custer State Park found that only

Table **138-3**

Semen Evaluation of Bison Bulls Relative to Age

Age (Months)	Animals Tested	Satisfactory (%)	Unsatisfactory (%)
18 to 24	404	72.28	27.72
24 to 36	107	93.46	6.54
36+	53	94.34	5.66

36.2% were rated satisfactory, despite the lower acceptable motility of 30%. This difference could be due to a variety of circumstances, including nutrition, body weight, differing bloodlines, different operator standards, or other factors.

AGE OF BULLS

Most producers use bulls between 2 and 7 years of age because older animals become difficult to manage and tend to be aggressive toward both humans and younger bulls, especially during the rut. If all bulls are of similar age, above about 5 years, they tend not to cede dominance, and fence damage frequently occurs. If a bull battery consists of animals of staggered ages, this problem does not occur.

BULL-COW RATIOS

Most producers run bison in multisire mating mobs with a bull-cow ratio between 1:10 and 1:15. There are no objective data to support these numbers, but they have no doubt developed from long practice. There appear to be no data that critically test the point at which calving seasons may be prolonged, or percentage of cows calving decline due to an inappropriately low ratio. Some producers, especially those that manage their herds to produce meat for the farm gate sale business, use higher ratios (up to 1:35) that create a prolonged calving season up to 3 or more months in duration.

PADDOCK MANAGEMENT

Management systems vary widely from extensive to intensive. In extensive systems the animals may be run in mobs of several hundred, being moved from large paddock to large paddock every few days.[15] In intermediate and mixed farming systems some degree of supplementary feeding is used, usually in winter.[16] Bison are also managed intensively, with year-round feeding.[17]

In those operations in which bulls are separated from cows for part of the year, the males are generally turned in with the females in late May or early June. The full rut does not start until mid to late July, and extends through August. There do not appear to have been any studies reported on the so-called "ram effect" in bison. Rutting behavior tails off sharply by September, as by then most cows have conceived. However, calves have been born in almost all months of the year, although calving in January, February, and the first half of March is considered exceedingly rare.

REPRODUCTIVE BEHAVIOR

Female bison are gregarious and live in groups with calves and immature individuals throughout the year. When a female is in estrus a male develops a tending bond with her that may last from a few hours to several days.[8,18,19,20] Bison are not harem breeders, and competition among males may be high because only a relatively small number of cows are in heat at one time.

Observation of breeding groups has shown that females may show typical signs of estrus that include a swollen vulva, raised tail-head, and close association with the bull. Bulls demonstrate typical flehmen reactions when tending a cow, and have been seen to actively discourage a cow that is coming into estrus when already tending a cow that is in a more advanced estrous state. Cows also exhibit flehmen toward one another, and it has been suggested that they use this behavior to stimulate the onset of estrus and synchronize the timing of copulation.[21]

During the tending period, which may last from a few hours to several days, the bull and cow adopt a position alongside one another, both facing in the same direction, and if a cow attempts to move away, the bull may swing his head across and intercept her.[18] It has been reported that 95% of bison females copulate only once per estrus period or, if more than once, with only one male.[1]

Aggressive interaction among bulls increases as they mature, and has been reported to peak between 8 and 11 years of age.[22] Commercial ranchers seldom keep bulls up to such a mature age, mainly because they become difficult to manage.

In free-ranging, mixed-age groups immature bulls may not get the chance to breed, because cows are tended and bred by older animals. However, they do breed opportunistically, often without showing any prolonged tending behavior.[22] During the early and middle parts of the rut mature bulls are the most aggressive, but younger bulls become increasingly aggressive as the rut progresses and mature bulls lose condition. If mature bulls are excluded from ranched herds, younger bulls show aggression throughout, but may be more reluctant to engage in behaviors involving risk, such as fighting.[22] For this reason most ranchers do not use fully mature bulls, but tend to use those under 5 years of age. However, a single mature bull in a breeding mob may prevent competition among the younger males while ensuring that they have opportunities to breed.

Mounting among bulls (Buller syndrome) has been observed among bulls up to 4 years of age and is commonly seen in mixed-age bull groups.[19]

PREGNANCY DIAGNOSIS

This subject is covered more fully in Chapter 139.[23] Techniques include rectal palpation, ultrasound imaging, serum sampling for progesterone or pregnancy-specific protein, and fecal sampling for steroid metabolites.

Pregnancy diagnosis is not widely practiced by bison ranchers, although there is no doubt that it would reveal deficiencies unrecognized by ranchers.[2] Both mummified fetus and freemartins have occasionally been recognized.

CALVING MANAGEMENT

There are reports of the killing of newborn calves by young bison bulls, although it is not known if this is due to curiosity or aggression. However, in intensive or semi-intensive operations young bulls should probably be removed from groups of calving cows.

Calving Behavior

Calving in bison is usually rapid, with calves weighing from 18 to 27 kg (about 4.5% to 7% of maternal body weight). They are unusually precocious.[20] The choice of calving site appears to depend on the type of habitat, and perhaps on herd size. In large groups on open grassland, calving commonly occurs within the group, with no attempt to search for isolation. Calving in denser habitat usually occurs in isolation.[10,20]

In general, bison cows have few calving problems. When they do have problems, they are difficult to deal with, and the outcome is seldom good.

One of the first signs of impending labor is that the cow will hold her tail elevated and well away from the vulval opening. The cow may also grunt, roll, paw the ground, or kick at her belly.

As parturition progresses, the cow will usually begin to strain while in a standing position, with her back humped. Shortly after the cow begins to strain, the amnion appears. Occasionally bison cows will deliver their calves standing, but in most cases the cow will lay down on her side with her feet extended. As she strains the cow will often lift her head and look backward. When the calf starts to emerge, the cow will frequently elevate her upper back leg. As soon as the calf is born, the cow will stand over it and lick it continually until it stands and nurses.

Dystocia

Because of the relatively small size of bison calves in relation to their dams dystocia is fairly uncommon, but certainly not unknown. Two causes of dystocia that appear to be of growing concern in the bison industry are fetal oversize and overfat dams.

Fetal Oversize

Within the bison industry, oversized calves have not been a major cause of calving problems in the past. However during the last few years, oversize calves have become a more common cause of dystocia in bison as some producers have followed beef industry management practices and selected on production parameters such as weaning weights, yearling weights, and 2-year-old weights.

Overfat Cows

Free-ranging bison cows lose weight during the winter months, and generally are thin when they enter the calving season. Ranched bison are generally provided with better quality feed than would be available under free-ranging conditions. Some bison producers that experience calving problems may have cows that are overfat at calving season. Target BCS should not exceed 2.5.

Management of Dystocia

It is difficult to make broad recommendations about how long to leave a calving bison cow before trying assist her. Obvious malpresentations require early intervention. In other instances it is not as easy to differentiate between a cow that requires assistance and one that is just taking a long time with a normal delivery.

In one study of free-ranging bison, bison cows took from 18 to 197 minutes to deliver their calf after the amnion was observed. They took from 1 to 40 minutes to deliver their calf after the calf's feet were first visible.[20] If these numbers are applied to farmed animals, it would be recommended that bison cows be allowed a minimum of 3.5 hours to deliver their calf after the amniotic sac is observed, and a minimum of 40 minutes once the calf's feet are observed.

A key component of calving management is that bison cows may delay labor for long periods if they are disturbed. If an observer interrupts the labor process and causes the cow to get up, she will delay the delivery of her calf. Observation of calving bison should be done from a long distance. Binoculars or a spotting scope are vital tools for the bison manager.

The incidence of dystocia in bison cows is low, and bison cows are often able to deliver calves that are in abnormal positions. In particular, presentations in which both back feet are presented first are not uncommon, and cows should be able to deliver such calves without any problems.[10] For these reasons bison producers often allow calving cows a considerable amount of time to work things out before they consider providing assistance.

Some bison producers make a deliberate decision not to interfere in dystocia cases at all. If a cow cannot deliver her calf unassisted, she is considered to be a cull animal and is shot, as leaving her would become a welfare issue.

The major goal of providing assistance to bison cows with dystocia is to save the cow, as the calf is likely to be dead. A major problem that then ensues is the capture and restraint of the cow. Because calving cows have a larger flight zone than noncalving cows, plenty of time should be taken to bait the cow or to slowly and calmly work her into a handling facility.[10] In most cases of calving trouble the calf will be dead before the cow has been identified as having a problem. If the calf is not dead, it will usually not survive the cow being aggressively chased into a handling facility. If the calf is dead, then time is not as critical, and the cow can be left for a period of up to 6 or 8 hours. It is important, however, not to leave the cow for too long as dehydration of the calf and the uterine environment will create serious problems that may make assisted delivery extremely difficult or cause toxemia in the cow.

If a cow must be tranquilized in the field, appropriate drugs must be used. The cow is likely to be stressed, especially if she has been chased, and the use of xylazine alone is unlikely to be effective. Drug combinations involving opioids and xylazine, or xylazine and Telazol are likely to be required.[24]

Once the cow is restrained in a squeeze or is tranquilized, assistance can be provided. Using sound principles of careful manipulation, and great care with the uterine wall, which may be fragile if assisted delivery has been delayed, the calf should be removed from the uterus as quickly as possible to minimize the amount of time the cow spends in the squeeze. The longer she stays in the squeeze the less likely will be her chance of survival.

Bison cows will often lie down if they are held within a squeeze for any time longer than a few minutes. If a hydraulic squeeze is not available, ropes can be tied under the cow's belly to act as a sling that will hold the cow up during the procedure.

If the malpresentation cannot be corrected by manipulation, a fetotomy is an option, but long procedures will reduce the cow's chance of survival.

Cesarean sections should be considered as a last resort. If the cows survive, the chances of them breeding back are limited.

Because of the very aggressive nature of newly calved bison cows, it is difficult to "mother up" calves that have been manually delivered, and we are unaware of any successful mothering up of calves and cows after cesarean section.

GESTATION LENGTH

Based on 261 known calving dates based on observed copulations, the length of gestation ranges from 277 to 293 days.[1]

There is good evidence to show that gestation length in bison varies with nutritional condition as well as date of breeding. Furthermore, primiparous females have longer gestations that multiparous ones (mean 288 versus 280 days).[1] Animals in poor condition will mate later than those in optimal condition, and will have longer pregnancies. However, females in good condition, bred after the median date, will reduce their gestation lengths by an average of 5.9 days, suggesting that they do so in order to synchronize births with other females in the herd.[1]

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CHAPTER 139

Pregnancy Determination and Fetal Aging of Farmed Red Deer, Wapiti, and Bison

PETER R. WILSON and JERRY C. HAIGH

Early pregnancy detection in wapiti, red deer, and bison can be accomplished by rectal palpation, rectal ultrasound examination, serum estrone sulfate (E_1S) concentrations, cross-reactivity with bovine pregnancy-specific protein (PSPB), and possibly, fecal hormone evaluation. Pregnancy diagnosis late in gestation is useful to determine if there have been fetal losses from scanning early in gestation. This may be performed by rectal manual or ultrasound examination, transabdominal ultrasound, or hormonal measurements, udder palpation, or observation. In red deer it is also possible to palpate a late-term fetus by abdominal ballottement. The principal requirement is that handling facilities and animal behavior be appropriate to ensure that the animals do not damage themselves, the operator, or the equipment while the examination is being conducted.[1]

MANUAL EXAMINATION

The simplest and least expensive method is rectal palpation, which can only be accomplished in animals large enough to permit the insertion of a hand. It is a relatively easy procedure in wapiti if restraint is adequate, but unless the operator has small hands (surgical glove size 6 or less) the red deer is difficult or impossible to palpate because of its smaller size. The variety of cross-bred animals on farms must be evaluated on an individual basis, and decisions on the advisability of rectal examination should be based upon both human hand size and the experience of the operator. Manual examination of bison for pregnancy determination is governed by the quality of the restraint method.

The cervix of the nulliparous red deer and wapiti is about 5 cm long and 1.5 cm in diameter. This part of the tract enlarges slightly during the first pregnancy and the cervix of multiparous wapiti females is about 5 cm in diameter and 10 cm in length.[1]

The uterus of nonpregnant wapiti lies almost entirely within the pelvic cavity. The yearling nonpregnant uterus is small, with a horn diameter of about 1.5 cm and a length of 8 to 10 cm. Enlargement in the pregnant horn can readily be detected manually by 42 days after conception, at which time it lies over the pelvic brim. At this time the uterus may be about 20 to 25 cm in length, and the first two of the enlarging placentomes on the dorsolateral surface of each horn can be felt. At this stage the wapiti fetus is about 24 mm in length, and can often be felt by finger-tapping of the fluid-filled uterus. The crown-rump (CR) length of the red deer fetus is about 19 mm at this stage of gestation. The fetal membrane slip technique has also been described. By 75 days of gestation the uterus has considerably enlarged, its weight has pulled it down into the abdominal cavity, and the fetus may be as much as 30 cm in length.[2] By 100 days of pregnancy it may be possible to determine only that the cervix lies over the pelvic brim, and that the uterus is out of reach, as it can be difficult for the operator to insert an arm past the elbow, and for some individuals even past the middle of the forearm, even in wapiti of 300 kg body weight.

The reproductive tract of the bison female is readily examined per rectum, in which criteria used have been adopted from cattle.[3,4] A small number of the animals may adopt a "dog-sitting" posture in the chutes, which delays proceedings.

ULTRASOUND

Transrectal Approach

Transrectal ultrasound examination, using a 5-MHz linear transducer on a rigid extender, has proved to be a highly reliable method of pregnancy diagnosis in red deer.[5,6] Although pregnancy confirmation is by observation of the fetal membrane, placentomes or fetal anatomy, the principal challenge for the ultrasonographer is definitive identification of the nonpregnant uterus.[6] A "nil diagnosis" is advised for any animal in which neither a pregnant nor a nonpregnant uterus can be detected, and these animals should be returned for repeat examination.

The fetal heartbeat can first be seen as early as 22 days of gestation in some wapiti, and by 24 days in all.[7] Pregnancy should therefore not be affirmed before 24 days, although other signs, such as the presence of an amniotic vesicle, may be seen before this time.[6] A cut-off of 28 days for confirmation of pregnancy by ultrasound, and a "nil diagnosis," with a second examination to follow, has been suggested as standard procedure for any animal in which a nonpregnant uterus cannot be detected.[8]

For the most accurate estimation of conception date in red deer it is recommended that ultrasonographic pregnancy diagnosis be carried out between approximately 35 and 60 days of gestation. At this time, amnion dimen-

sions and CR length can be measured, and estimates based on these have standard errors of about 2 days.[5] Although conception dates of red deer can be estimated upon measurements made after 60 days of pregnancy, the standard error of the estimate is greater. Up to 13 fetal and uterine dimensions may by used to estimate conception date.[5] Note, however, that fetal aging, while providing an accurate estimation of conception date, cannot be used to accurately predict the calving date of an individual deer, because of the normal variation in gestation length described earlier. However, fetal aging can give an accurate prediction of the pattern of calving of a herd, because variation in gestation length of an individual is random about the mean. Defining the herd pattern of conception and therefore calving dates is known as "pregnancy profiling" in New Zealand.[8]

As pregnancy advances and the uterus moves over the pelvic brim, fetal anatomy may be difficult to scan after about 110 to 130 days in red deer. In wapiti, by 100 days, it may not be possible to determine pregnancy if the uterus has advanced into the abdominal cavity and lies between the posterior face of the rumen and the ventral abdominal muscles, where a probe, even with an extension handle attached, cannot reach it.[1]

Transabdominal Approach

Ultrasound can also be used via the external, transabdominal approach in the later stages of gestation in red deer, by applying the probe to the relatively hairless area immediately adjacent to the udder. A 3.5-MHz sector scanner has been used in New Zealand. This technique can also be used in reindeer in later gestation, but the heavy winter coat limits the site for application of the scanner. A hair-free site between the teats has been used in Norwegian reindeer.[9]

Ultrasound is also becoming established as a useful tool for pregnancy diagnosis in bison, but no data on fetal sizing have been reported.[4]

HORMONE MEASUREMENTS

Hormonal evaluation has been used to determine pregnancy in free-ranging populations of wapiti. Serum progesterone (P4) concentrations are raised during pregnancy, but because progesterone concentrations also rise during the estrous cycle, and the levels during pregnancy and the luteal phase may overlap. Therefore, because wapiti and red deer are polyestrous until after midwinter, if progesterone concentrations are to be used for pregnancy determination in that period, they must be used either in conjunction with other methods of diagnosis, or sampling must be repeated at intervals shorter than the generally accepted estrous cycle length of 18 to 21 days. If carried out after the spring equinox when normal estrous cyclicity has ceased in nonpregnant animals, elevated progesterone concentration in a single sample is diagnostic.[1]

Similarly, hormonal metabolite concentrations of ungulates have been measured in urine and feces to determine reproductive status. Although this technique has not been demonstrated in wapiti or red deer, it has the potential to be of benefit as a noninvasive method of diagnosis. It is most likely to be used in free-ranging populations rather than farmed animals. It is also used in zoo animals when handling facilities may not be available, or caretakers may wish to make determinations in a noninvasive manner.[10]

Estrone sulfate (E_1S) concentrations have also been used in the determination of pregnancy. It has been shown to be 100% effective in distinguishing pregnancy when hinds are more than 100 days pregnant.[11]

PREGNANCY-SPECIFIC PROTEIN

A pregnancy-specific protein in red deer cross-reacts with the pregnancy-specific protein of bovine origin (PSPB).[12,13] Wapiti pregnancy-specific protein has been characterized and shown to have similar properties to that of cattle.[14] The protein, produced by the binucleate cells of the trophoblast, is produced only during pregnancy, and in red deer it is reliably present 33 days after conception.[12]

When PSPB levels were compared to direct uterine examinations, lowering the criterion used to indicate pregnancy from 95% to 93%, binding of wapiti (elk) antiserum to bovine PSPB improved overall pregnancy detection accuracy from 94% to 96% and reduced the rate of false positive tests from 15% to 3%.[15]

Bovine pregnancy-specific protein assays have also been used for pregnancy determination in bison, and have proved to be as accurate as rectal palpation.[3] For more details on pregnancy diagnosis in bison, see Goodrowe and associates.[10]

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CHAPTER 140

Brucellosis in Specialized Livestock

LORRY B. FORBES

The genus *Brucella* consists of six formally recognized species of bacteria found in terrestrial mammals (*abortus*, *melitensis*, *suis*, *ovis*, *canis,* and *neotomae*) and a proposed species recently described in marine mammals.[1,2] These gram-negative bacteria are the causative agents of brucellosis in animals. A number of species have more than one biologic variant. There are seven recognized biovars of *B. abortus*, five of *B. suis,* and three serovars of *B. melitensis*.[3] Two distinct strains of *B. canis* have been reported, and there is evidence to suggest more than one biovar for the marine isolates.[2,4] *Brucella ovis* and *B. neotomae* have no reported variants. The preferred hosts for individual species are cattle (*B. abortus*), goats and sheep (*melitensis*), sheep (*ovis*), dogs (*canis*), and wood rats (*neotomae*). *Brucella suis* is notable because it has a wider range of normal hosts than the other species of *Brucella* and these hosts are associated with specific biovars. Thus, the normal hosts for *B. suis* biovars 1 and 3 are swine; for biovar 2, swine and the European hare; for biovar 4, reindeer and caribou; and for biovar 5, rodents. The host specificity of *Brucella* is not always absolute, and it appears that in some cases it is an artifact of normal host range. When a normal and a potential host come in contact through translocation, game farming, extension of agricultural zones into wildlife habitat, or for other reasons, there is an opportunity for interspecific transfer to occur. Examples of this are the spread of *B. abortus* from cattle to bison and *B. ovis* from sheep to red deer. The following sections describe the disease by host for bison, elk, reindeer, caribou, red deer, white-tailed deer, mule deer, moose, and other deer species. Table 140-1 lists the species and biovars of the genus *Brucella*, the normal hosts, and the specialized livestock species that have been infected with brucellosis either in the field or experimentally.

AMERICAN BISON (*Bison bison*)

Brucellosis in bison probably originated from contact with European cattle in the 19th and early 20th centuries.[5] The disease is self-sustaining in bison herds, as evidenced by longstanding infection of relatively isolated populations in Wood Buffalo National Park in Canada, and the greater Yellowstone area of the United States.[5,6] *Brucella abortus* biovar 1 (typical and urease negative) and biovar 2 have been recovered from bison.[7,8] Clinical signs, pathogenesis, and epidemiology of bison brucellosis are similar to those seen in cattle, but serologic tests and vaccines developed for use in cattle do not always give equivalent responses in bison and this needs to be considered in diagnosis and control.

Epidemiology

The epidemiology of brucellosis in bison is not well documented, but the similarities of the clinical disease in bison and cattle and the ability of the disease to sustain itself in infected bison herds suggests that epidemiologic

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Table **140-1**

Normal and Specialized Livestock Hosts for the Species and Biovars of the Genus *Brucella*

			SPECIALIZED LIVESTOCK HOST	
***Brucella* Species**	**Biovars**	**Normal Hosts**	**Experimental Infection (Host and Infecting Biovar)**	**Field Infection (Host and Infecting Biovar)**
B. abortus	1–6, 9	Cattle	Bison (1) Elk (1) White-tailed deer (1) Moose (1) Axis deer (1) Mule deer (unknown)	Bison (1, 2) Elk (1, 4) Moose (1) White-tailed deer (unknown)
B. melitensis	1, 2, 3	Goats Sheep		
B. suis	1	Swine		
	2	Swine European hare		
	3	Swine		
	4	Reindeer Caribou	Bison (4) Moose (4) White-tailed deer (4)	Moose (4)
	5	Wild rodents		
B. ovis	1	Sheep	Red deer (1) White-tailed deer (1)	Red deer (1)
B. canis	1	Dogs		
B. neotomae	1	Desert wood rats		
Marine sp. (unnamed)	1 or more	Seals Whales Sea otters		

aspects of the disease are similar, if not identical, in both species.[9] Oral infection is the primary route of infection. Aborted fetuses, placentas, and uterine fluids from infected bison cows can contain high numbers of *B. abortus*, and persistent vaginal shedding may occur for several weeks post partum. Licking and nosing of aborted materials or the genital regions of postabortion females provides ample opportunity to transmit the disease and these behaviors have been observed in domestic bison. In cattle, persistent shedding of *B. abortus* may occur after abortion or following subsequent normal calvings, but successive abortions are uncommon. There are no field infection data for bison, but a vaccine strain (*B. abortus* strain 19) was recovered from a bison cow after her second abortion following vaccination, suggesting that both persistent shedding and successive abortions may occur.[10]

Venereal transmission rarely occurs in cattle, and infected bulls are considered a negligible risk factor. However, transfer of the organism directly into the uterus via artificial insemination will cause disease.[11,12] This is because the vaginal environment is not conducive to survival of *Brucella* sp., whereas the uterine environment provides optimal growth conditions. Bison bulls infected with *B. abortus* are similar to bovine bulls and can shed the organism in semen. This is likely not a significant factor in transmission. Two experimentally infected bison bulls shown to be shedding *B. abortus* in semen were housed with six susceptible bison cows for 10 months. Four cows were successfully bred by one of the bulls and none of the cows or their calves were positive on culture or showed seroconversion.[13]

In cattle, superovulation, artificial insemination, and embryo transfer have been used to produce disease-free calves from brucellosis-infected cows. Similar procedures have been used with infected donor bison cows to produce calves bacteriologically negative for brucellosis.[13] The use of infected cows and bulls for breeding purposes is generally discouraged, but these methods provide an option for the preservation of valuable germ plasm in diseased populations of bison.

Brucella organisms are commonly present in the milk of infected cattle and have been shown to be present in the milk of some experimentally infected bison cows for 3 weeks.[9] Live calves born to infected dams are likely to ingest *Brucella* organisms in milk, but the dose received and the effect of anti-*Brucella* colostral antibodies are complicating factors. The importance of this route of infection among bison is unknown. Meconium from aborted calves contain *Brucella* organisms, as do feces from calves nursing infected dams, and these may be a factor in transmission under conditions of close confinement.[9]

In areas cohabited by bison and cattle, it should be remembered that cattle and bison strains are indistinguishable and that interspecific transmission is possible.

Cattle have been infected with *Brucella* from bison under both field and experimental conditions and *B. abortus* strain 2308 has been successfully transmitted from infected cattle to noninfected bison cohabiting the same pen.[14–16] Experimental infection of six bison with the reindeer strain of brucellosis (*B. suis* biovar 4) showed that this strain is not pathogenic for bison and that reindeer cohabiting range with bison are unlikely to pose a risk.[17]

Pathogenesis

The pathogenesis of brucellosis is similar among hosts despite the different species and biovars found in the genus *Brucella*.[18] Infection proceeds through four stages: mucosal infection, infection of local lymph nodes, bacteremia and secondary localization, and reproductive tract localization. Invasion of the mucosa occurs following exposure and elicits an acute inflammatory response in submucosal tissues characterized by increased numbers of mononuclear, polymorphonuclear, and eosinophilic leukocytes; plasma cells; and focal lymphoid aggregates. *Brucella* sp. are facultative intracellular parasites and likely resist intracellular killing by releasing of 5′-GMP and adenine from their surface, which interferes with degranulation (phagosome-lysosome fusion) and inhibits myeloperoxidase–hydrogen peroxide–halide bacteriocidal activity. Their survival within host leukocytes offers additional protection against other humoral and cell-mediated bacteriocidal responses. Organisms escaping the submucosal response travel to local lymph nodes via lymphatic drainage where they cause enlargement due to lymphoid and reticuloendothelial hyperplasia and the infiltration of inflammatory cells. Survival and multiplication of the organisms in the regional lymph nodes eventually results in spillover into the vascular system and hematogenous spread to other tissues, most notably lymphoid tissues, uterus, and mammary gland in females and testes, epididymis, and accessory sex organs in males. Bones and synovial membranes are less frequently involved and a variety of other tissues and organs can harbor the organism, particularly during the bacteremic phase.

Brucella organisms have an affinity for the placenta, and are found in high numbers in chorionic trophoblasts.[18] Chorionic trophoblasts produce a variety of hormones and secretory proteins and appear to provide the factors necessary to stimulate rapid growth of *Brucella* sp. Localization in the reproductive tract was originally attributed to the presence of erythritol in these tissues, but subsequent studies showed that erythritol was not a critical requirement. Infection may be associated with minimal placentitis or with severe placentitis characterized by widespread destruction of placentomes and fetal membranes. Abortion is a frequent sequela, regardless of the severity of placentitis, and is caused by *Brucella* lipopolysaccharides and lipid A (endotoxins) present in the uterine environment. The exact mechanism causing abortion is not completely understood and is probably due to a combination of factors. Both placental and fetal progesterone are required to maintain pregnancy in cattle and sheep during the second half of gestation. Virulent strains of *Brucella* destroy chorionic trophoblasts in vitro, and if this occurs in vivo, it may disrupt the progesterone balance. Bovine and ovine fetuses infected with highly pathogenic strains of *Brucella* have elevated cortisol levels and this may result in decreased progesterone, increased placental estrogen production, subsequent increases in endometrial prostaglandin (PGF_2), and induction of parturition. This fetal effect does not occur consistently with strains of low virulence (RB51, strain 19). Interestingly, abortions have been associated with *B. abortus* strain 19 vaccination in bison indicating that *Brucella* strains of low virulence for cattle are not necessarily of low virulence for bison.[10]

The pathogenesis of mammary gland infection is not well described, but is associated with focally distributed interstitial infiltrates of plasma cells, lymphocytes, macrophages, and variable numbers of neutrophils.[18] A variety of pathologic changes associated with inflammatory responses can also occur following localization in the male reproductive tract. Orchitis, epididymitis, and seminal vesiculitis have been described, and lesions may range from severe necrotizing pyogranulomatous orchitis to mild seminal vesiculitis.[19]

Localization in the joints probably occurs following traumatic injury or endotoxin-mediated changes to capillary permeability resulting in hemorrhage into the joint. Arthritic lesions in bison were characterized by serous arthritis with lymphoplasmacytic infiltrates and hemosiderin-laden macrophages in synovium and proliferation of synovial villi.[7] Foci of necrosis and mineralization occurred within the synovium and joint capsule, and there was an infiltrate of epithelioid macrophages, macrophages, and lymphocytes and proliferation of fibroblasts.[7]

Clinical Signs

Abortions are readily induced by experimental infection of bison with cattle strains of *Brucella* and abortions due to brucellosis have been described in both wild and ranched bison.[9] Based on the limited evidence available, abortions in bison, as in cattle, tend to occur in the last 3 months of pregnancy. Gross lesions of the fetus are seldom observed. Histopathologically, a mild bronchointerstitial pneumonia characterized by neutrophils, mononuclear cells, and degenerate leukocytes in bronchioles and alveoli has been described in aborted fetuses and infected neonates, as have splenic infarction, splenic necrosis, and purulent nephritis.[8,20] Retained placentas occur in experimentally infected, vaccinated, and field-infected bison cows.[8,9] Metritis in a field-infected bison was associated with a purulent vaginal discharge and necrotic placenta, debris, and purulent exudate in the uterine lumen, all of which were cultured positive for *B. abortus*, and a recently aborting bison cow had a purulent endometritis and necropurulent placentitis.[8,21]

Orchitis, epididymitis, and seminal vesiculitis have been described in bison that were serologically or bacteriologically positive for *B. abortus*.[9] Grossly enlarged scrotums were a common clinical sign. Visible lesions ranged from scrotal sacs filled with purulent exudate and caseous testicular remains to thickened epididymal tissue containing foci of yellowish purulent exudate. Gross lesions

are not always present, but microscopic lesions in the epididymis, seminal vesicle, and ampulla consisting of lymphoplasmacytic infiltrates with neutrophils in the interstitium and glandular lumens have been described. Based on the limited data, the severity of testicular lesions probably varies with the dose and duration of infection and there is no doubt that reproductive performance is affected in some cases.

B. abortus has been recovered from arthritic joints and hygromas in wild male bison.[7] Affected joints may be grossly distended and mobility may be inhibited. Arthritic lesions included loss of articular cartilage with eburnation and lysis of bone, synovial villous hyperplasia, pannus formation, and thickening of joint capsules. Large amounts of translucent viscous yellow fluid containing flecks of debris were present in the joint space and distended tendon sheaths. One bull had a suppurative exudate in the stifle joint. The severity of lesions, and subsequent degree of lameness, likely varies according to the dose and duration of infection.

Diagnosis

Brucellosis should be considered as a differential diagnosis in cases of late stage abortion, testicular enlargement, low conception rate, or lameness of unknown etiology. On a herd basis, brucellosis is easily diagnosed using serologic tests designed for use in cattle. On an individual animal basis, variations in test sensitivity and specificity from that seen in cattle may compromise test interpretation. Culture and recovery of the organism from suspected cases provides the only true definitive diagnosis.

Serology

A number of serologic tests are available for use in cattle, and most, if not all, can be used for bison. These tests include the buffered plate agglutination test (BPAT), rapid screening test, brucellosis card test (BCT), standard tube agglutination test (SAT) , rivanol test, complement fixation test (CFT), particle concentration fluorescence immunoassay (PCFIA), indirect and competitive enzyme immunoassays (iELISA, cELISA), and most recently, the fluorescence polarization assay (FPA).[9,22] The sensitivity and specificity of serologic tests used for bison vary widely, and validation has been hampered by the relatively low number of samples available for study. It has been suggested that serologic responses on some tests are slower to develop in bison than in cattle, and that no serologic test is reliable during the first 8 weeks after infection.[16] In general, the tests reliably recognize bison that are positive on culture (sensitivity from 75% to 100%), but do not consistently predict culture results (specificity 36% to 100%).[9] The BCT is a readily available and easily conducted field assay and has been used as the sole test to successfully eradicate brucellosis from an infected bison herd. Recent validation work has shown an iELISA, cELISA, and FPA test to have a sensitivity of 96.3%, 96.3%, and 96.3% and a specificity of 97.6%, 94.1%, and 97.6%, respectively, making them the tests of choice.[22] The FPA can also distinguish between strain 19 vaccine infection and field infection.[22] However, it should be remembered that in the early stages of infection, vaccination and field infection titers may be indistinguishable.

For practical purposes, the deficiencies of individual serologic test procedures can be overcome by using a battery of different tests and, if necessary, conducting retests at 3- to 4-week intervals. Strong positive reactions on serologic assays are reliable indicators of exposure but do not necessarily mean that individual bison are harboring significant numbers of the organism. Low level reactions must be interpreted with caution, particularly if they occur in only a few animals in otherwise serologically negative herds. For both herd and individual animal testing, the strength of a presumptive diagnosis of brucellosis increases with the number and magnitude of observed serologic responses.

Bacteriology

Recovery of *Brucella* organisms is required for a definitive diagnosis.[3] The success of bacteriologic culture is dependent on the number, type, and quality of samples submitted, the experience of the diagnostic laboratory, and the duration of infection in the animal sampled. *Brucella abortus* is widely distributed in lymph nodes and organs of infected animals, but not necessarily in all these tissues. In chronic infections, only a few tissues may remain infected and these may contain small numbers of the organism. A large selection of samples is required to maximize the sensitivity of bacteriologic culture. Tissues associated with abortion are important for culture. Samples of choice are fetal stomach contents, fetal lungs, and placenta. Fetal spleen, liver, kidneys, and lymph nodes and vaginal exudates are also good candidates. Milk is a useful sample for bacteriologic culture, but shedding of the organism in milk is often erratic. Mammary tissue provides more reliable results. In the male, semen can be cultured, but infected bulls do not always shed in semen. Bacteriologic culture of testicle and epididymis will give more reliable results, particularly if abnormalities are present. The accessory reproductive glands are of lower priority, but should be sampled if lesions are present. In both sexes, lymph nodes draining the head (retropharyngeal, parotid, mandibular) and inguinal region (internal iliac, supramammary in female; superficial inguinal in male) are tissues of choice for culture, but additional body lymph nodes such as the mediastinal, bronchial, mesenteric, hepatic, suprascapular, prefemoral, and popliteal will enhance the sensitivity of bacteriologic culture. Fluid or suppurative material from affected joints of animals with lameness or joint enlargements should be aseptically collected and cultured. Occasionally the organism will be present in nontypical locations, such as bones or kidneys, and any lesions in animals suspected of having brucellosis should be sampled.

Samples for bacteriologic culture should be chilled to 4°C as soon as possible after collection and processed within 72 hours. If processing will be delayed, samples can be frozen at –20°C and stored for several months. *Brucella* sp. are slow growing and compete poorly with contaminant bacteria on commonly used media. In most cases, successful isolation requires specialized media and procedures; therefore, samples should be submitted to

a laboratory familiar with the isolation of *Brucella* sp.[3] Confirmation and biotyping of suspected *Brucella* isolates require specialized chemical reagents, bacteriophages, and antisera and are usually performed by a national or international reference laboratory.[3] Molecular procedures are being developed for the identification and biotyping of *Brucella* isolates.

Treatment and Control

There is no effective and economic antibiotic treatment for brucellosis in cattle, and none has been described for bison. *Brucella abortus* in bison is a reportable disease under national animal health programs and control is by whole herd depopulation or by quarantine, serologic testing, and slaughter.

Vaccines

Brucella abortus strain 19 is a live vaccine that is commonly used in cattle but offers limited protection against infection and abortion in bison. In addition to this, antibodies produced following vaccination with strain 19 are indistinguishable from those produced following field infection and are a confounding factor in serologic diagnosis. In one study, 58% of pregnant bison aborted following strain 19 vaccination, and one of these bison aborted a strain 19 infected calf during her subsequent pregnancy as well.[10] In another study, 61% of vaccinated bison became infected and 33% aborted following challenge with a virulent strain of *Brucella* (*B. abortus* strain 2308).[10] Female bison vaccinated as 8-month-old calves and challenged with *B. abortus* strain 2308 during their second trimester of pregnancy had an infection rate of 91% and an abortion rate of 75%.[23] Seventy-three percent of pregnant bison vaccinated with strain 19 remained positive on a least one serologic test 10 months after vaccination.[10]

Brucella abortus strain RB51 is also a live vaccine and was developed from a mutant strain of *B. abortus* strain 2308 that does not produce the lipopolysaccharide O side chain characteristic of the smooth brucellae. In cattle, this strain protects at least as well as strain 19, does not cause abortion, and does not induce the formation of detectable antibodies on standard serologic tests for brucellosis.[24,25] This vaccine has been approved for use in cattle in the United States. Strain RB51 vaccine is less pathogenic for bison than strain 19 vaccine and is cleared by 30 weeks without shedding or lateral transmission following calfhood vaccination.[26,27] As in cattle, RB51 in bison stimulates a specific cell-mediated immunity that does not interfere with standard serologic tests for brucellosis.[27] Pregnant bison may abort following vaccination using cattle doses, and vaccination of adult bull bison may result in transient shedding of strain RB51 in semen.[28,29] In a study using calfhood vaccination with RB51, 15% (4/27) of vaccinated bison aborted following challenge with *B. abortus* strain 2308 as compared to 62% (4/7) of control subjects.[30] There was a significant reduction in the recovery of strain 2308 from the uterus and mammary gland of the RB51-vaccinated animals. In the field, this would reduce the likelihood of both horizontal transmission via fluids associated with abortion or parturition and vertical transmission via milk. Based on these data, it has been suggested that calfhood vaccination with RB51 would be beneficial in reducing transmission of brucellosis among bison. The duration of immunity is unknown, and initial studies on the safety of revaccinating pregnant bison previously vaccinated as calves are not conclusive.[31]

Zoonotic Potential

All field strains of *Brucella* from bison and *B. abortus* strain 19 vaccine are pathogenic for humans. Vaccine strain RB51 is a rough mutant strain and is likely of low virulence for humans. This is based on the low pathogenicity of other rough species (*B. ovis* and *B. canis*) for humans and case reports of accidental exposure.[32] Until more field data on RB51 are available, it would be prudent to consider it a human pathogen.

NORTH AMERICAN ELK (*Cervus elaphus* subspp.)

Brucellosis in elk (wapiti) caused by *B. abortus* originated from contact with infected cattle and bison during the early 20th century.[33] *Brucella abortus* biovars 1 and 4 have been recovered from elk, and clinical signs and pathogenesis of elk brucellosis are similar to those seen in cattle and bison.[33] Confinement in pens or population concentrations on managed winter feeding grounds during periods when abortions are occurring provide the necessary conditions for transmission. Currently, brucellosis in elk due to *B. abortus* occurs only in the wild elk of the greater Yellowstone area of the United States, and control programs using vaccination and feeding ground–habitat modifications are being attempted.[33]

Epidemiology

Oral exposure is the primary route of infection. Under natural conditions female elk isolate themselves at calving and rapidly consume the products of parturition.[34] This strategy appears effective in preventing intraspecific transmission in wild populations. Elk on feedgrounds or in confinement have been observed to smell, lick, and consume products of parturition from other elk, including aborted fetuses and this provides ample opportunity to transmit the disease. Seventeen of 18 unexposed cow elk and 6 of 6 unexposed bull elk became infected following natural exposure to artificially infected elk, indicating that elk are highly susceptible to infection; and 33% to 100% of naturally or experimentally infected elk were shown to either abort or produce nonviable calves, demonstrating a mechanism for the dissemination of large numbers of organisms into the environment.[33,35] Three successive abortions were reported in one elk cow.[35] In addition to placental and fetal tissue, chorioallantoic fluid is considered a significant source of environmental contamination.[35] Vaginal shedding of *B. abortus* has been reported for up to 17 days following abortion or birth of nonviable calves, and positive vaginal cultures were obtained for up to 9 days following normal parturition in an infected elk cow.[35] The duration of infection under natural conditions is unknown. *B. abortus* has been recovered from experimentally infected elk 56 months after inoculation. Latent infections lasting 23 months (no clinical or serologic evidence of disease) were

observed in two elk cows after which they developed serologic titers and aborted, and latent infections probably occur in some calves born to infected dams.[35]

B. abortus has been recovered from the epididymis, seminal vesicles, ampullae, and semen of experimentally infected elk, but natural breeding of infected bulls to noninfected cows failed to transmit the disease.[35] Venereal transmission is not a significant epidemiologic factor in elk, and this is assumed to be for the same reasons as described for bison and cattle.

Brucella organisms were present in the milk of one of five lactating infected elk, indicating that some calves ingest *Brucella* organisms in milk.[35] The dose received and the effect of anti-*Brucella* colostral antibodies are complicating factors, and the importance of this route of infection among elk is unknown. Meconium from three nonviable calves contained *Brucella* organisms, but feces from calves nursing infected dams did not, and the authors concluded that environmental contamination from elk calf feces was not important.[35]

Historical evidence suggests that appropriate calving management under confinement conditions or the opportunity for female elk to exhibit normal isolation behavior at parturition is sufficient to prevent transmission of disease. Brucellosis was confirmed in the elk and bison of the greater Yellowstone area in the early 1900s, and it is generally agreed that it was present for some time before that. Between 1892 and 1967, over 14,000 wild elk from this area were either given or sold to a variety of private, corporate, and government organizations in 38 states and 3 countries.[36] It is likely that some of these animals had brucellosis, yet there are no reports of this disease in any of the destination herds. This is likely due to a combination of factors. The number of infected elk transported was probably low. Animals for public display were a novelty at their destination and were closely monitored. Under these conditions it is reasonable to assume that transmission was limited by the prompt removal of aborted fetuses or dead calves, and in many cases, by separation of the valuable pregnant females from the rest of the herd. Elk translocated for restocking or otherwise released under range conditions were able to mimic normal wild behavior at parturition and thus effectively block transmission. In contrast, the wild elk of the greater Yellowstone area have been concentrated in high numbers on managed winter feeding grounds. Under these conditions, detection and isolation of aborting females, effective removal of fetuses, and cleanup of contaminated sites is not possible prior to exposure of other elk, and this appears sufficient to maintain the disease in these herds.

Cattle have been infected with *B. abortus* from elk under experimental conditions.[33] In areas cohabited by bison, cattle, and elk, it should be remembered that the *B. abortus* strains infecting these species are indistinguishable and that interspecific transmission is possible (although unlikely unless calving or abortion occurs during cohabitation).

Pathogenesis

The pathogenesis of *B. abortus* infection in elk is as described for cattle and bison. Infection proceeds through four stages: mucosal infection, infection of local lymph nodes, bacteremia and secondary localization, and reproductive tract localization. A detailed description is contained in the preceding section for bison.

Clinical Signs

Abortions in the last 3 months of pregnancy and nonviable calves are the most common clinical signs in both naturally and experimentally infected elk. Fifty-four percent (7 of 13) naturally exposed elk and 61% (40 of 60) experimentally infected elk aborted or had nonviable calves.[33,35] Three consecutive abortions were observed in one elk cow; however, the frequency of repeat abortions under field conditions is unknown.[35] Gross lesions of the fetus have not been reported. Retained placentas occur in cattle and bison, but have not been observed in elk.[35] Infertility in cows is not a common sequela.

Clinical signs and gross lesions of orchitis, epididymitis, and seminal vesiculitis have not been observed in elk, although *B. abortus* was isolated from the epididymis, seminal vesicles, and ampullae of 4, 7, and 6 of 17 experimentally infected bulls, respectively.[35] All tissues of 6 of the 17 infected bulls in this group were negative on culture. There are no confirmed reports of infertility in bull elk associated with *B. abortus* infection.

Carpal bursitis (hygroma) was the second most frequently observed sign in infected elk.[35] It occurred in 12 of 65 elk with experimental infections of at least 1 month duration, although it is usually associated with more chronic infections. Distention was grossly apparent in some cases, and visible only on necropsy in others. This condition was not associated with lameness and often resolved spontaneously. Inflammation of synovial membranes and distention of joint cavities and tendon sheaths, often associated with severe lameness, occurred in 13 of 65 infected elk examined at necropsy.[35] *Brucella abortus* was isolated from the anterior and posterior fetlock, anterior and posterior flexor tendon sheath, and carpal, stifle, and hock joints of these animals.

Diagnosis

Brucellosis in elk caused by *B. abortus* should be considered as a differential diagnosis in cases of late stage abortion or lameness of unknown etiology. On a herd basis, brucellosis is easily diagnosed using serologic tests designed for use in cattle. On an individual animal basis, variations in test sensitivity and specificity from that seen in cattle may compromise test interpretation. Culture and recovery of the organism from suspected cases provides the only true definitive diagnosis.

Serology

A number of traditional serologic tests designed to detect brucellosis in cattle have been used for elk. The complement fixation test (CFT) and agglutination tests such as the standard plate agglutination test (SPT), buffered plate agglutination test (BPAT), and rivanol test (Riv) have been used successfully to detect infected elk.[37] Specificity data for elk are lacking for most of the traditional tests and therefore the incidence of false positive reactions is unknown. The CFT was the most sensitive of these tests,

but the anticomplementary activity frequently observed in serum from cervidae can cause inconclusive results. Indirect and competitive enzyme immunoassays (iELISA, cELISA), and the fluorescence polarization assay (FPA) have recently been validated for detection of *B. abortus* infection in elk and were compared with the CFT.[38] The sensitivity and specificity of the iELISA, cELISA, and FPA exceeded 99% for each test, and the FPA was able to correctly distinguish 84% of 55 *B. abortus* strain 19 vaccinated elk (4 months after vaccination) from infected elk. Test results using a commercially available cELISA kit were 98.6% specific for elk 6 months to 2 years after vaccination with *B. abortus* strain 19 and 100% sensitive in detecting infected animals.[39] However, in elk tested 15 to 43 days after vaccination, specificity dropped to 88%, and the authors suggest that the test not be used within 2 months of strain 19 vaccination.

In general, strong positive reactions on serologic assays are reliable indicators of exposure, particularly if reactions occur in a battery of tests and are present in a number of animals in a herd. However they do not necessarily mean that individual animals are harboring large numbers of the organism. Low level reactions must be interpreted with caution, particularly if they occur in only a few animals in otherwise serologically negative herds. For both herd and individual animal testing, the strength of a presumptive diagnosis of brucellosis increases with the number and magnitude of observed serologic responses.

Bacteriology

Recovery of *Brucella* organisms is required for a definitive diagnosis.[3] As for other hosts, the success of bacteriologic culture is dependent on the number, type, and quality of samples submitted; the experience of the diagnostic laboratory; and the duration of infection in the animal sampled. *Brucella abortus* is widely distributed in lymph nodes and organs of infected animals, but not necessarily in all these tissues. In chronic infections, only a few tissues may remain infected, and these may contain small numbers of the organism. A large selection of samples is required to maximize the sensitivity of bacteriologic culture. Sampling is as previously described for bison except that seminal vesicles and ampullae should be included for male elk.

Treatment and Control

There is no effective and economic antibiotic treatment for brucellosis in elk. *Brucella abortus* in elk is a reportable disease under national animal health programs and eradication is possible using a number of strategies including herd depopulation, isolation of dams at parturition, and serologic testing and slaughter. Since the only known infected elk herds are the wild herds in the greater Yellowstone area, these strategies are not economically or politically feasible. Instead, a program of vaccination and altered winter feeding management has been suggested to control the disease.[33]

Vaccines

Brucella abortus strain 19 is a live vaccine that offers incomplete protection against infection and abortion in elk.[40] Twenty-seven percent of mature elk vaccinated with a cattle dose of strain 19 aborted. Reduced doses (3.7×10^7 to 7.6×10^9) did not cause abortion or result in bacteremia, but 45% (19 of 42) of vaccinated elk and 69% (9 of 13) of nonvaccinated elk became infected following challenge. Sixty-two percent (23 of 37) of strain 19 vaccinates calved successfully as compared to 33% (3 of 9) of nonvaccinates. In a similar study using elk vaccinated as calves with strain 19 and subsequently challenged as pregnant adults, 29% of vaccinates (13 of 45) calved successfully as compared to 5% (2 of 44) of nonvaccinated control animals.[41] However, there was no difference in the number of infected fetuses/calves between the two groups. Simulations indicate that field vaccination of feedground elk with strain 19 is unlikely to reduce infection rates below 20%, and this is supported by limited field data from one herd showing a reduction in the rate of serologic reactions from approximately 46% to approximately 25% after 8 to 9 years of vaccination.[33,42]

Brucella abortus strain RB51, described above for bison, has been assessed as both an oral and intramuscular vaccine in elk.[43–47] Seroconversion does not occur on standard serologic tests for brucellosis, and bulls do not have grossly detectable reproductive lesions or shed the organism in semen. However, in various studies using pregnant elk previously vaccinated with strain RB51, 4 of 8, 5 of 7, 14 of 16, 12 of 16, 16 of 16, and 13 of 14 elk aborted or produced nonviable calves following challenge with *B. abortus* strain 2308.[43,44,46,47] Consequently, strain RB51 vaccine is not recommended for use in elk.

Zoonotic Potential

All field strains of *Brucella* from elk and *B. abortus* strain 19 vaccine are pathogenic for humans. Vaccine strain RB51 is a rough mutant strain and is likely of low virulence for humans. This is based on the low pathogenicity of other rough species (*B. ovis* and *B. canis)* for humans and case reports of accidental exposure.[32] Until more field data on RB51 is available, it would be prudent to consider it a human pathogen.

REINDEER AND CARIBOU (*Rangifer tarandus* subspp.)

Brucellosis in reindeer and caribou is caused by *Brucella suis* biovar 4 and is endemic in the circumpolar Arctic except for Scandinavia, Greenland, and the Canadian Arctic east of Hudson Bay. There is debate as to whether rangiferine brucellosis spread to North America across the Bering Sea land bridge during the last ice age, or if it was introduced in imported Russian reindeer around 1900.[48,49] The pathogenesis of rangiferine brucellosis is similar to that seen with brucellosis in other species but is characterized by a more pronounced granulomatous response, resulting in an increased severity and variety of lesions. Epidemiologically, the disease is similar to that seen in other species. It has been suggested that rangiferine brucellosis has a cyclic component and that latent infection may play an important role, but the existence and significance of this has yet to be determined.

Epidemiology

Oral exposure is the primary route of infection. Aborted fetuses, placentas, and uterine fluids from infected reindeer or caribou contain high numbers of *B. suis* biovar 4.[49,50] Infected females had 10^3 to greater than 10^5 organisms per gram of uterine tissue for at least the 4 weeks following calving or abortion.[50] Licking and nosing of aborted materials or the genital regions of females following abortion or parturition provides ample opportunity to transmit the disease. Females usually abort during the first parturition following infection, but successive abortions are uncommon.[49]

Reindeer and caribou bulls frequently have extensive reproductive tract pathology and shed the organism in semen. However, there is no evidence of venereal transmission, presumably for the same reasons previously described for bison and cattle. In one experiment, a female reindeer inoculated intravaginally with *B. suis* biovar 4 at 3.5 months of gestation calved successfully and had a live calf the following year as well.[48]

Mammary tissue or milk from infected females contained 10^3 to greater than 10^5 organisms per gram for at least 4 weeks following abortion or parturition.[50] Live calves born to infected dams are likely to ingest *Brucella* organisms in milk, but the dose received and the effect of anti-*Brucella* colostral antibodies are complicating factors. The importance of this route of infection in rangiferine species is unknown.

Cattle can become infected with *B. suis* biovar 4 if they are in contact with infected reindeer that are calving. Four of 8 cattle housed with 17 infected reindeer were cultured positive for *B. suis* biovar 4 58 days after the reindeer were removed from their pen.[50]

Pathogenesis

Suppurative lesions are observed more frequently and in a wider variety of tissues in reindeer and caribou than in other species infected with brucellosis.[49,51] The reason for this enhanced inflammatory response is unknown. Otherwise, the pathogenesis of brucellosis in reindeer and caribou is as previously described for bison. Infection proceeds through four stages: mucosal infection, infection of local lymph nodes, bacteremia and secondary localization, and reproductive tract localization.

Clinical Signs

Clinical signs usually involve the reproductive and musculoskeletal systems, and less commonly a variety of other tissues and organs.[49,51–53] Infected females may abort during the last 3 months of pregnancy, or give birth to weak or nonviable calves. Retained placentas and metritis may be present, and mastitis with abscessation of the mammary gland is frequently observed. In some cases of mastitis normal mammary tissue is almost entirely displaced by purulent exudate.

Infected males may have inflammatory lesions in the testicles, epididymides, seminal vesicles, and ampullae. In some cases, the scrotal sac may be grossly distended by swollen testicles or epididymides or by purulent exudate associated with orchitis and epididymitis. Reproductive capacity will be significantly reduced in males with severe bilateral orchitis and sterility is probably the final outcome.

Lameness associated with bursitis or synovitis may be present alone or in conjunction with other clinical signs. Carpal and fetlock joints are the most frequently involved, and affected joints may be grossly distended by serofibrinous or thick purulent exudate. Extension may occur into tendon sheaths and hooves and osteoarthritis may be present in chronic infections.

Abscesses in *Brucellosis*-infected reindeer and caribou often contain an odorless, thick, light green purulent material. In addition to the tissues listed above, abscesses or granulomas have been described in lymph nodes, liver, kidney, spleen. and subcutaneous tissues.

Diagnosis

Brucellosis in reindeer or caribou should be considered as a differential diagnosis in cases of late-stage abortion, testicular enlargement, low conception rate, lameness of unknown etiology, or the presence of suppurative lesions in any tissue. On a herd basis, brucellosis is easily diagnosed using serologic tests designed for use in cattle. On an individual animal basis, variations in test sensitivity and specificity from that seen in cattle may compromise test interpretation and there is evidence to suggest that some female reindeer and caribou with established infections may not be detected at certain stages of the disease.[50,54] Recovery of the organism from suspected cases provides the only true definitive diagnosis.

Serology

A number of serologic tests developed for use in cattle, including the buffered plate agglutination test (BPAT), rapid screening test, brucellosis card test (BCT), standard tube agglutination test (SAT), rivanol test, mercaptoethanol test, antiglobulin test (AGT), and complement fixation test (CFT), have been used successfully to detect rangiferine brucellosis.[50,55] In general, these tests have adequate sensitivity, particularly in acute infections, but fail to detect some animals with established infections. These animals may not be producing sufficient antibody for detection (latent infection), or the *B. abortus* antigen used in these tests (dominant A surface antigen) is not sufficiently sensitive to detect low levels of antibody produced in response to infection with *B. suis* biovar 4 (dominant M surface antigen). The CFT was the most sensitive of these tests, but the anticomplementary activity frequently observed in serum from cervidae may prevent testing of some samples. Specificity data for reindeer and caribou are not available for most of the traditional tests, and therefore the incidence of false positive reactions is unknown.

An indirect and competitive enzyme immunoassay (iELISA, cELISA) and a fluorescence polarization antibody test (FPA) have recently been validated for the detection of brucellosis in various cervids including *B. suis* biovar 4 infection in reindeer and caribou.[38] The sensitivity and specificity of the iELISA, cELISA, and FPA ranged between 98% and 100% for these tests. The FPA was recommended

as a test of choice because it was technically simple, adaptable for field use, and relatively inexpensive to perform.

For practical purposes, the deficiencies of individual serologic test procedures can be overcome by using a battery of different tests and, if necessary, conducting retests at 3- to 4-week intervals. In general, strong positive reactions on serologic assays are reliable indicators of exposure, particularly if reactions occur in a battery of tests and are present in a number of animals in a herd. However, they do not necessarily mean that individual animals are harboring large numbers of the organism. Low level reactions must be interpreted with caution, particularly if they occur in only a few animals in otherwise serologically negative herds. For both herd and individual animal testing, the strength of a presumptive diagnosis of brucellosis increases with the number and magnitude of observed serologic responses.

Bacteriology

Recovery of *Brucella* organisms is required for a definitive diagnosis. As for other hosts, the success of bacteriologic culture is dependent on the number, type, and quality of samples submitted, the experience of the diagnostic laboratory, and the duration of infection in the animal sampled. *Brucella suis* biovar 4 is widely distributed in lymph nodes and organs of infected reindeer and caribou, but not necessarily in all these tissues. In chronic infections, only a few tissues may remain infected and these may contain small numbers of the organism. A large selection of samples is required to maximize the sensitivity of bacteriologic culture. Sampling is as previously described for bison. In addition to tissues with lesions and a wide selection of lymph nodes, seminal vesicles and ampullae should be sampled from male reindeer and caribou. Suppurative material from any location should be cultured.

Treatment and Control

There is no effective and economic antibiotic treatment for brucellosis in reindeer and caribou.

Vaccines

Brucella suis biovar 4 (killed) vaccine is the only vaccine shown to be useful for reindeer.[56] A number of other vaccines have been tried, including *B. melitensis* H38 (killed), *B. abortus* strain 45/20 (killed), *B. abortus* strain 19 (live), and *B. abortus* strain RB51 (live). Four of five reindeer vaccinated with *B. melitensis* strain H38 (killed) and two of three control reindeer either aborted or had nonviable fawns following challenge with *B. suis* biovar 4.[57] One of six reindeer vaccinated with *B. abortus* strain 45/20 (killed) and two of four control reindeer either aborted or had nonviable calves following challenge.[55] Seven of 11 pregnant reindeer vaccinated with *B. abortus* strain 19 (live) and challenged with *B. suis* biovar 4 either aborted, had nonviable calves, or were nonpregnant at the end of the experiment.[58] *Brucella abortus* strain 19 vaccine alone induced abortions, caused long-term infections, and was shed into the environment in sufficient quantities to cause seroconversion in naive reindeer.[59] *Brucella abortus* strain RB51 does not interfere with standard serologic tests for brucellosis, which is a desirable characteristic for control programs. In a preliminary safety trial using RB51, 54.5% (12 of 22) pregnant reindeer cows aborted following vaccination, and this vaccine is not recommended for use in reindeer until additional evaluations are completed.[60]

Zoonotic Potential

Brucella suis biovar 4 can cause severe disease in humans and occurs most frequently in people from Arctic areas who are associated with the slaughter and consumption of caribou or reindeer. *B. abortus* strain 19 vaccine is pathogenic for humans. Vaccine strain RB51 is a rough mutant strain and is likely of low virulence for humans. This is based on the low pathogenicity of other rough species (*B. ovis* and *B. canis*) for humans and case reports of accidental exposure.[32] Until more field data on RB51 are available, it would be prudent to consider it a human pathogen.

RED DEER (*Cervus elaphus* subspp.)

Naturally occurring brucellosis in red deer caused by *Brucella ovis* was first reported in New Zealand in 1996.[61] The causative organism is identical to that causing brucellosis in sheep and the disease in red deer is similar, if not identical, to *B. ovis* infection in sheep. It primarily affects the male reproductive organs and can cause decreased fertility or sterility. It is believed that the disease spread from sheep to red deer, and this is supported by experimental transmission of *B. ovis* from infected rams to noninfected stags.[62] At present, this disease occurs only in New Zealand.

Epidemiology

Oral exposure of stags to infected semen during the rut is the primary mode of transmission.[63] Behavioral studies comparing the homosexual activity of rams and stags indicated that mounting and preputial sniffing and licking occurred less frequently in stags than in rams, but with sufficient frequency to facilitate transmission.[64] It was concluded that sniffing or licking of semen from the preputial area, or from the perineum after one stag has mounted another is a more important route of transmitting disease among stags than rectal copulation. It is presumed that spread from rams to stags takes place in the same fashion. Stag to stag and ram to stag infection studies have shown that animals must be confined together to facilitate transmission.[62,64] The disease was not transmitted under conditions where infected and noninfected stags were rotated through the same pasture or where infected and noninfected stags were separated by a common fence.[65]

Brucella ovis has not been identified as a source of reproductive failure in hinds. In sheep, ewes develop transient infections and can mechanically transmit the disease to clean rams, but do not transmit infection from one season to the next.[66] Experimental studies indicate a

similar situation likely exists with red deer.[67] Under field conditions, the rate of infection for hinds following mating with an infected stag, and the frequency of disease transmission to other stags during the same estrus, the next estrus, or in the following rut appears low, but requires further study.[68,69]

Pathogenesis

The pathogenesis of *B. ovis* infection in red deer is similar to that described for other hosts.[18] Infection proceeds through four stages: mucosal infection, infection of local lymph nodes, bacteremia and secondary localization, and reproductive tract localization. *Brucella ovis* infection is unique because lesions are usually limited to the male reproductive tract. The pathogenesis in stags is probably identical to that described for rams.[66] Localization of *B. ovis* in the epididymis causes perivascular edema and accumulation of lymphocytes, monocytes, and neutrophils in peritubular tissue. The tubular epithelium is eventually destroyed by bacteria or the inflammatory response resulting in an extravasation of spermatozoa. The host response to the spermatozoa leads to the formation of large spermatic granulomas that may completely block the epididymis and cause testicular degeneration and fibrosis.

Clinical Signs

Sperm samples may contain abnormal sperm and sperm may have decreased motility.[70] In severe cases, there may be frank pus in the sperm sample. Fertility may be decreased and some stags may be infertile. Testicular or epididymal lesions may be present, but they are generally small and difficult to detect on scrotal palpation. Clinical signs have not been reported in hinds.

On postmortem examination, suppurative or caseous testicular lesions up to 5 mm in diameter were observed in 10 of 15 stags cultured positive for *B. ovis*.[71] Granulomas and interstitial lymphoid cells were frequently seen on histologic examination.

Diagnosis

Brucellosis in red deer caused by *B. ovis* should be considered as a differential diagnosis in cases of male or apparent female infertility, orchitis, or epididymitis, or where abnormal sperm samples are observed. On a herd basis, the disease is easily diagnosed using serologic tests designed for *B. ovis* in sheep. Serologic tests to detect antibodies to *B. abortus, B. suis,* and *B. melitensis* will not detect antibodies to *B. ovis*. This is because the smooth (A and M) surface antigens of *B. abortus, B. suis,* and *B. melitensis* do not cross-react with antibodies produced against the rough (R) surface antigen of *B. ovis*. Serologic titers have been reported to recede more quickly in infected red deer than in sheep, and this may compromise test interpretation.[64] Culture and recovery of the organism from suspected cases provides the only true definitive diagnosis.

Serology

The complement fixation test for *B. ovis* is the recommended test and is considered highly sensitive in detecting acute infections. It had a specificity of 98.9% based on 183 known negative animals.[64] The gel diffusion test and ELISA test for *B. ovis* may also be used but may be less sensitive.[72] Because extensive validation data are not yet available for these tests, repeat testing, use of a battery of tests, and bacteriologic examination of semen would be prudent in questionable cases, such as a single serologically reacting animal in an otherwise negative herd.

In general, strong positive reactions on serologic assays are reliable indicators of exposure, particularly if reactions occur in a battery of tests and are present in a number of animals in a herd. However, they do not necessarily mean that individual animals are harboring large numbers of the organism. Low level reactions must be interpreted with caution, particularly if they occur in only a few animals in otherwise serologically negative herds. For both herd and individual animal testing, the strength of a presumptive diagnosis of brucellosis increases with the number and magnitude of observed serologic responses.

Bacteriology

Recovery of *Brucella* organisms is required for a definitive diagnosis. As for other hosts, the success of bacteriologic culture is dependent on the number, type, and quality of samples submitted, the experience of the diagnostic laboratory, and the duration of infection in the animal sampled. Based on the disease in sheep, *B. ovis* is probably widely distributed in lymph nodes and organs of infected deer, but not necessarily in all these tissues. In experimental infections of sheep it has been recovered from the liver, kidney, spleen, testicles, epididymides, vesicular glands, bulbourethral glands, ampullae, and iliac, prescapular, precrural, submaxillary, parotid, and retropharyngeal lymph nodes.[66] Since the target organs for *B. ovis* in deer appear to be the epididymides and accessory sex glands, they are the samples of choice for bacteriology, and semen is a useful sample to culture from a live animal. In chronic infections, and cases in which semen culture is negative but the animal remains seropositive, only a few tissues may remain infected and these may contain small numbers of the organism. A large selection of samples will maximize the sensitivity of bacteriologic culture. Sampling in addition to semen sampling should be done as previously described for bison.

Treatment and Control

Test, slaughter, and herd management techniques similar to those used for sheep in New Zealand have been recommended for the control and eradication of *B. ovis* in red deer.[63] Antibiotic treatment has not been evaluated. In sheep, streptomycin plus tetracycline, and dihydrostreptomycin plus oxytetracycline have been used to treat *B. ovis,* and similar regimens may prove useful for salvaging valuable stags.[69] Vaccination using live *B. melitensis* strain Rev. 1 is 74% to 100% protective against *B. ovis* infection in 3- to 5-month old rams but has not been advocated for use in red deer.[69]

Zoonotic Potential

Brucella ovis is not pathogenic for humans.

WHITE-TAILED DEER (*Odocoileus virginianus*) AND MULE DEER (*Odocoileus hemionus*)

Brucellosis does not naturally occur in white-tailed or mule deer. Experimental infections with *B. abortus, B. ovis,* and *B. suis* biovar 4 (rangiferine brucellosis) have been established in white-tailed deer.[73–76] Mule deer have been exposed to *B. abortus, B. suis,* and *B. neotomae* under experimental conditions, but infections have not been confirmed bacteriologically.[77] The possibility of interspecific transmission exists in places where white-tailed deer or mule deer are closely confined with other infected host species.

Epidemiology

White-tailed and mule deer are widely distributed across North America and have cohabited range with infected cattle and bison for decades. A single bacteriologically confirmed field case of *B. abortus* in a white-tailed deer was reported in 1964, but extensive serologic surveys before and after that time have consistently failed to provide evidence of infection with *B. abortus, B. suis,* or *B. melitensis* in deer populations.[78,79]

The few serologic surveys to detect *B. ovis* in white-tailed and mule deer were negative and there is no clinical or pathologic evidence of brucellosis caused by *B. ovis* in these species. In New Zealand, transmission of *B. ovis* from sheep to red deer has been observed, and once established in red deer, intraspecies transmission was similar to that observed in sheep. Experimental infections using *B. ovis* indicate that a similar situation is possible with white-tailed deer and contact spread of *B. ovis* from an infected to a noninfected buck in the same pen has been documented.[75]

White-tailed deer have been experimentally infected with *B. suis* biovar 4.[76] This biovar of *Brucella* normally occurs in reindeer and caribou in Arctic areas where geographic isolation provides a natural barrier to field exposure. Transmission of *B. suis* biovar 4 to a moose (*Alces alces*) under field conditions has been documented, and presumably transmission from reindeer and caribou to other deer species could occur if ranges overlap.[80] There is evidence to support latent infection in reindeer, and this could pose a transmission risk in places where game farmed reindeer and white-tailed/mule deer cohabit the same range.

There is no evidence to indicate that brucellosis is maintained in wild white-tailed or mule deer populations. Oral exposure is the primary route of infection and the browsing characteristics of white-tailed and mule deer under natural conditions probably limit their exposure to *Brucella* sp. by preventing contact with contaminated forage.[79] Under conditions of close confinement, it would be prudent to assume that the mechanisms for transmission of *B. abortus* and *B. suis* biovar 4 are similar to those described for bison, elk, and reindeer, and for *B. ovis*, similar to those described for red deer.

Pathogenesis

The limited experimental data indicate that the pathogenesis of brucellosis in white-tailed and mule deer is similar that described for bison, elk, and reindeer (*B. abortus*, *B. suis* biovar 4) or red deer (*B. ovis*).[73–77] Refer to the preceding sections for bison, elk, reindeer, and red deer.

Clinical Signs

Brucella abortus

No definitive clinical signs were reported in four white-tailed deer or the single mule deer experimentally infected with *B. abortus*, although there was a transient febrile response from days 8 to 15 in the mule deer.[73,74,77]

Brucella suis

Clinical signs were not observed in any of five male white-tailed deer experimentally infected with *B. suis* biovar 4, and no gross lesions were observed at postmortem examination 6 weeks after infection.[76] Four of the five deer were cultured positive for *B. suis* biovar 4 at necropsy. The organism was isolated from lymph nodes, liver, and spleen, but not from testes and epididymides. There are no data on clinical signs in females, intraspecific transmission, or long-term infections.

Brucella ovis

The clinical signs and lesions observed in nine male white-tailed deer experimentally infected with *B. ovis* were similar to those observed in sheep and red deer (see discussion of red deer) infected with the same organism.[75] Varying degrees of epididymal enlargement occurred, and in some cases there was adherence of the tunic to the epididymides and grossly visible cavitations containing creamy white exudate. Lesions were not always present. *Brucella* ovis was recovered from the normal appearing epididymides of a white-tailed buck on PID 429 and positive semen cultures were obtained on PID 102.[75] Intraspecific transmission occurred from an infected buck to an noninfected buck cohabiting the same pen.[75] There are no data on clinical signs in females; however, based on observations in other species infected with *B. ovis*, they are likely to be minimal.

Diagnosis

Brucellosis caused by *B. abortus*, *B. suis* biovar 4, or *B. ovis* is rare, if it occurs at all, in white-tailed and mule deer. However, if clinical signs include one or more of the following: abortion, stillbirth, abnormal sperm, testicular lesions, lameness, mastitis, or multiple granulomatous lesions in various tissues, and the etiology is unknown, brucellosis should be considered. When reviewing the history it is important to determine if affected deer were in contact with known host species for brucellosis such as bison, elk, cattle, reindeer, caribou, red deer, or domestic sheep.

Serology

Serologic tests for cattle have been used to detect antibodies to *B. abortus*, *suis,* or *melitensis* in white-tailed and mule deer but little or no validation data exist for their use in these species. Under experimental conditions standard plate and tube agglutination tests for cattle used on sera from white-tailed deer infected with *B. abortus* had serologic profiles similar to that seen in infected cattle, and the complement fixation test (CFT) was better than the serum agglutination test (SAT), card test, and rivanol test (Riv) for detecting white-tailed deer infected with *B. suis* biovar 4 (four of four bacteriologically positive deer detected).[73,74,76] A number of serologic assays have been evaluated in other cervids, such as elk and reindeer, and are good candidate tests for use in white-tailed and mule deer. The complement fixation test and agglutination tests such as the standard plate agglutination test (SPT), buffered plate agglutination test (BPAT), and rivanol test (Riv) were used to detect elk infected with *B. abortus*.[37] Indirect and competitive enzyme immunoassays (iELISA, cELISA), the fluorescence polarization antibody test (FPA), and the CFT were validated for detection of *B. abortus* infection in elk and *B. suis* biovar 4 infection in reindeer and caribou and had a sensitivity and specificity of ≥99% for each test.[38] It should be remembered that the anticomplementary activity frequently observed in serum from cervidae can cause inconclusive results on the CFT.

Serologic tests for cattle will detect antibodies to *B. abortus, suis,* and *melitensis*, but not to *B. ovis*. If testicular abnormalities are present and contact with sheep (or red deer) may have occurred, the complement fixation test for *B. ovis* should be conducted in addition to the other tests. In nine white-tailed bucks infected with *B. ovis*, the CFT correlated well with isolation results from the semen or epididymides, particularly in older bucks.[75] This is supported by data from red deer in which the CFT for *B. ovis* is considered highly sensitive in detecting acute infections and had a specificity of 98.9% based on 183 known negative animals.[64] The gel diffusion test and ELISA test for *B. ovis* may also be used, but validation data are not available for these tests in white-tailed or mule deer. In questionable cases, such as a single serologically reacting animal in an otherwise negative herd, repeat testing, use of a battery of tests, and bacteriologic examination of semen are advisable.

In general, strong positive reactions on serologic assays are reliable indicators of exposure, particularly if reactions occur in a battery of tests and are present in a number of animals in a herd. However, they do not necessarily mean that individual animals are harboring large numbers of the organism. Low level reactions must be interpreted with caution, particularly if they occur in only a few animals in otherwise serologically negative herds. For both herd and individual animal testing, the strength of a presumptive diagnosis of brucellosis increases with the number and magnitude of observed serologic responses.

Bacteriology

Recovery of *Brucella* organisms is required for a definitive diagnosis. As for other hosts, the success of bacteriologic culture is dependent on the number, type, and quality of samples submitted; the experience of the diagnostic laboratory; and the duration of infection in the animal sampled. *Brucella* organisms are widely distributed in lymph nodes and organs of infected animals, but not necessarily in all these tissues. In chronic infections, only a few tissues may remain infected, and these may contain small numbers of the organism. A large selection of samples is required to maximize the sensitivity of bacteriologic culture. Sampling is as previously described for bison except that seminal vesicles, bulbourethral glands, and ampullae should be included for white-tailed and mule deer bucks. Semen is a useful sample to culture, particularly if *B. ovis* is suspected.

Treatment and Control

Brucellosis in white-tailed and mule deer rarely, if ever, occurs under natural conditions, and no treatment or control programs exist. However, if these deer species are maintained in contact with infected hosts on managed ranges or under game farming conditions, interspecies transmission could occur. Control and treatment strategies would then be based on those developed for cattle, elk, and bison for *B. abortus*, reindeer for *B. suis* biovar 4, and red deer or sheep for *B. ovis*.

Zoonotic Potential

Brucella abortus and *B. suis* biovar 4 are pathogenic for humans. *Brucella ovis* is not a human pathogen.

MOOSE (*Alces alces*)

Experimental and field evidence indicate that *B. abortus* and *B. suis* biovar 4 cause a severe debilitating disease in moose, culminating in death.[80–86] Moose cohabiting areas with brucellosis-infected host species, such as cattle, bison, elk, reindeer, and caribou, are at risk of acquiring brucellosis. Live vaccines for brucellosis have not been tested on moose, and because of their apparent susceptibility to *Brucella* infection, inadvertent or purposeful exposure during routine vaccination programs for other species may cause clinical disease.

Epidemiology

Naturally occurring *B. abortus* infections in moose have been described on four separate occasions, three of which were associated with brucellosis-infected bison or cattle herds.[83–86]

Similarly, the single field case of *B. suis* biovar 4 infection in a moose was associated with infected caribou.[80] Moose and caribou are sympatric in northern areas of North America, and moose with antibodies to *Brucella* are occasionally found in these areas, further supporting occasional field exposure.[87] Moose are likely infected orally through the ingestion of forage or water contaminated with infected products of parturition from normal host species cohabiting the same range. The severe pathologic lesions observed in field and experimental infections and the low number and debilitated condition of field cases indicates that brucellosis in moose is probably

fatal and intraspecific transmission is rare or does not occur.

Pathogenesis

The pathogenesis of *B. abortus* and *B. suis* biovar 4 infection in moose is similar to that observed in the normal hosts for these species of *Brucella*, but the lesions are more extensive and severe.[81,82] The specific anti-*Brucella* cell-mediated immune response associated with protection in other hosts appears to be diminished in moose and results in high numbers of *Brucella* bacteria in tissues and an associated endotoxemia.[81] Experimentally infected moose had a prolonged bacteremic phase (≥166 days for *B. abortus* and ≥103 days for *B. suis* biovar 4) and their lymph nodes frequently contained greater than 4×10^4 bacteria per gram.[81,82] There are no data on the reproductive effects of brucellosis in moose, but considering their susceptibility, it is likely that abortion, orchitis, epididymitis, and other reproductive disorders can occur with a pathogenesis similar to that observed in other species. A variety of lesions occurred in moose experimentally infected with *B. abortus*.[81] Lymph nodes were consistently involved. They varied in size from grossly normal to markedly enlarged. Lesions ranged from mild to moderate follicular hyperplasia with low numbers of giant cells in the sinusoids and subcapsular sinuses to severe follicular hyperplasia, edema, congestion, fibrin deposits within distended subcapsular sinusoids, foci of hemorrhage and cortical necrosis, thrombi, and increased numbers of macrophages and giant cells. Fibrin and fibrous tags were often present on the pulmonary pleura, omentum, various abdominal organs, and in carpal joints. Liver enlargement sometimes occurred, and foci of necrosis and hemorrhage were often present on the capsule. Multiple small foci of necrosis in the hepatic parenchyma and cuffs of macrophages and lymphocytes around the portal triads were present on histologic examination in some cases. Carpal joints were the only affected joints. Lesions in the synovium included hyperemia, hemorrhage, and fibrosis, and fibrin on the surface. Villus hypertrophy and pannus formation were observed. Joint fluid varied from amber-colored to clear, yellow viscous fluid in increased volume. Similar carpal lesions were described in the single field case of *B. suis* biovar 4.[80]

Clinical Signs

Clinical signs did not occur in any of the three moose heavily infected with *B. abortus* and killed at day 70 (two moose) and day 166 (one moose) after infection, which was remarkable considering the extremely high tissue load of *Brucella* in these animals.[81] A fourth *B. abortus*–infected moose with a similar bacterial load exhibited weakness and depression for only 24 hours before dying of acute endotoxic shock 85 days after infection.[81] A single moose infected with *B. suis* biovar 4 was anorexic, depressed, and had a body temperature elevation from 38° to 39.5°C from day 42 to 58 after infection, after which it returned to normal.[82] In four field cases of brucellosis in moose the animals were described as thin, weak, and having abnormal behavior.[83–86]

Clinical Pathology

Clinical chemistry and hematology were not remarkable in four moose experimentally infected with *B. abortus* and did not predict the onset of clinical signs and death or indicate the persistent bacteremia present.[81] It was not until 24 hours before death that one of these moose had clinical pathologic values consistent with endotoxemia and systemic collapse. One week prior to death, this moose had a rise in white blood cell count from 6.2 to 11.5×10^9 cells/L, but the relative proportion of cells in the differential count remained unchanged and this was within the normal range for cattle. A similar rise was seen at day 55 in a single moose infected with *B. suis* biovar 4.[82] It was not associated with clinical signs and was maintained until the moose was killed on day 103.

Diagnosis

Brucellosis should be ruled out when sick, debilitated, or dead moose are encountered in areas where known host species for brucellosis occur. Currently available vaccines for brucellosis have not been evaluated for use in moose, and exposure to live vaccines for brucellosis, such as *B. abortus* strain 19, could cause clinical disease.

Serology

The common serologic tests used for cattle will detect *B. abortus* and *B. suis* biovar 4 infection in moose. Experimentally, there is individual variation in postexposure seroconversion, but by day 21 after infection most moose have a significant response using cattle criteria for interpretation.[81,82] One heavily infected moose did not have a positive CFT result until 43 days after infection.[81] Once seroconversion occurs, antibody titers are maintained at high levels on all tests.[81,82]

Bacteriology

Bacteriologic findings are as described for bison. The organism is present in large numbers in a variety of tissues and is readily cultured. Blood contains much lower numbers of bacteria than lymph nodes, but the prolonged bacteremia makes whole blood a useful sample for a definitive diagnosis of brucellosis in live moose.

Treatment and Control

Antibiotic treatment has not been attempted in moose and the commonly used cattle vaccines (strain 19 and RB51) have not been evaluated for efficacy in moose. Cattle vaccines have either caused disease or been ineffective when used in other species, and this makes them poor candidates for use in moose.[88] At present, the most effective control method would be to ensure that moose do not cohabit range with known infected host species.

Zoonotic Potential

Brucella abortus and *B. suis* biovar 4 are pathogenic for humans. The high bacterial load in the tissues of infected moose poses a significant zoonotic risk to persons

butchering or conducting postmortem examinations on these animals.

OTHER DEER SPECIES

Axis deer (*Axis axis*) appear highly susceptible to experimental infection with *B. abortus*.[89] Thirty days after infection four axis deer had positive results on serologic tests used for cattle, as well as widespread distribution of the organism in tissues, and in milk from a lactating female. The course of infection after 30 days is unknown, but the strong serologic response, systemic involvement, and lack of field survey evidence of disease despite opportunities for exposure suggest that the disease in axis deer may be similar to that seen in moose.

Brucella abortus was isolated from two of 126 roe deer (*Capreolus capreolus*) in Switzerland (reported in 1964), but there have been no reports of brucellosis in roe deer since that time.[90] Serologic surveys for brucellosis have included fallow deer (*Cervus dama*), Japanese deer (*Cervus nippon)*, Muntjac deer (*Muntiacus reevesi*), Chinese water deer (*Hydropotes inermis*), roe deer, pampas deer (*Ozotocerus bezoarticus*), and sambar deer (*Cervus unicolor*).[79,91,92] Titers were rarely demonstrated, and when they occurred, they were usually low. Many of the survey titers were probably nonspecific, as the surveillance tests used were designed for cattle and their specificity in deer is unknown. It is likely that most species of deer are susceptible to brucellosis if appropriate conditions of exposure are met, but these conditions and the progression of disease will likely vary. In places where deer cohabit areas with known infected hosts for brucellosis, abortion, infertility, stillbirths, weak calves, and testicular lesions may indicate brucellosis and should be considered in a differential diagnosis. A wide variety of other lesions, such as those described for reindeer and moose, may also be present and provide additional clinical evidence. It should be remembered that the current serologic tests for *B. abortus, suis*, and *melitensis* will not detect exposure to *B. ovis* and vice versa. In general, the serologic and bacteriologic tests for diagnosis are the same as those previously described for other cervids.

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CHAPTER 141

Other Reproductive Diseases of Deer and Bison

JERRY C. HAIGH

Comprehensive information about reproductive abnormalities in deer and bison is not yet available. In this chapter an attempt is made to collate reports form a variety of sources. Some information comes from published literature or the author's own records, but the advent of two list servers, Deermail and ElkMed, has allowed correspondence between farmers and scientists, and some of the information presented here was supplied by several correspondents. It is likely that as time progresses more conditions will be recognized.

INFERTILITY OR SUBFERTILITY

Infertility has been seen following recovery from epizootic hemorrhagic disease in white-tailed deer bucks. These animals also showed external evidence of infertility, as they did not clean the velvet from their antlers and essentially became physiologic castrates.[1]

A variety of sperm defects, similar to those in other species, have been detected in red deer, wapiti, and fallow deer, as well as bison, but there is little work on these defects and their possible relationship to infertility or subfertility.[2] A high proportion of knobbed acrosomes has been observed in a single infertile wapiti stag,[3] and a stump-tail defect that caused infertility and was carried in the female line has been seen, also in wapiti.[4]

Cases of subfertility or infertility have been observed in fallow bucks. In two cases only 5% to 10% of does joined produced fawns, and this was not associated with abortion or other disease entities.[5] Similar unexplained fertility reduction has been seen in white-tailed deer and wapiti. In a herd of white-colored red deer studied over 12 years reproductive rates were below 50%, which was considered to be related to inbreeding.[6]

CONGENITAL DEFECTS

Several congenital defects have been reported. Freemartinism has been reported in red deer, wapiti, and reindeer and in *Cervus* has become more prevalent since the advent of artificial breeding and the use of synchronization techniques that often lead to twinning.[7] Gonadal hypoplasia, brachygnathia, absence of active teats, and intersex cases have been reported. In female fallow and axis deer the following conditions have been seen: nabothian cysts in cervix blocking patency, cystic ovaries (parovarian cysts), pyometra, metritis, and mastitis.

In a 12-year study of red deer in the Czech Republic, and in numerous subsequent instances at the same facility, a variety of reproductive anomalies have been seen.[8,9] Many of these were thought to be associated with specific genetic anomalies in an inbred herd of white-colored red deer.[6,8,9] There were 56 perinatal deaths associated with 176 births, but in only 3.4% (6 animals) could morphologic defects be seen on gross examination. The malformations included anophthalmy (1 case), termination of the digestive tract into the vagina (1), undeveloped anus (1), and shortened legs to about 50% of normal height. There have been numerous cases of brachygnathia, which did not compromise survival.[10]

TRAUMA

A few cases of classic "broken penis" in wapiti, similar to the condition known in cattle, have been reported by clinicians subscribing to the "ElkMed and "Deermail" list servers.[11,12] In one of these, semen collection via electroejaculation was still possible.

Anal intromission during mating leading to death of the hind in one case, and prolonged evidence of pain in another, has been reported in wild red deer.[13] The author has records of a case in which a wapiti stag, penned with a group of four white-tailed deer females in a small enclosure, attempted to mate them and caused extensive internal trauma and death in each of them.[3]

An unusual incidence of rupture of the suspensory ligament of the udder occurred in three white-tailed deer that were held in a drop-floor chute for a few minutes shortly after their fawns were weaned. One animal died acutely after rupturing the mammary artery. In this animal and one other extensive hemorrhage undermined the skin on both hind legs below the stifle joints as far down as the hocks (Fig. 141-1). In the third animal the hemorrhage was not as severe.[3] Deer are routinely handled in such chutes for a variety of purposes, with no ill effects. It was hypothesized that in this case the animals struggled in the chute, thrashing their legs back and forward, damaging the distended udders, which had not been suckled for 24 hours.

In a 15-year study of penned red deer in the Czech Republic, infanticide by incoming males was demonstrated. It was considered that the males thereby improved the fecundity of the females that they were associated with because those females did not have to

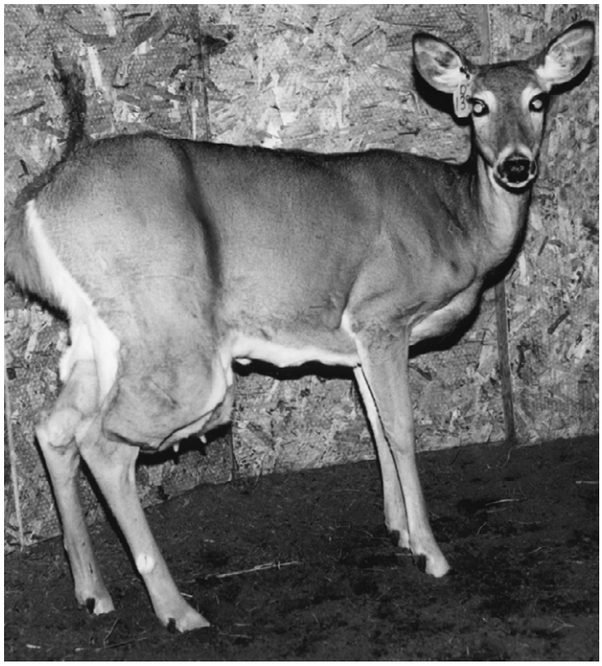

Fig. 141-1 Extensive undermining of the skin and damaged udder that included a ruptured suspensory ligament in a white-tailed deer.

nurse dependent young and so gained in body condition and had improved chances of conceiving during the rut.[14]

DISEASES OF THE REPRODUCTIVE TRACT

Two widely recognized sporadic conditions of wapiti, of unknown etiology, are ulceration of the vestibule and posterior vagina and ulceration of the prepuce[2] (Figs. 141-2 and 141-3). It is tempting to think that they may be related, and possibly even a venereal condition, but no confirmation of this hypothesis has been achieved. Attempts at isolation of several different pathogens have not provided a reliably reproducible etiologic agent.

Another theory of causation of this condition proposes that it may be due to chemical burning of the mucous membranes due to excessive protein in the diet and renal excretion of ammonia. The author has seen this condition in males that had been on a 13% protein diet for several weeks and considers this hypothesis of causation unlikely, but there is no clearly demonstrated etiology.

In the female the condition has most often been seen when animals are being synchronized for artificial breeding, and the lesions appear as shallow ulcers varying in size up to as much as 1 cm in diameter. They may be covered in a diphtheritic membrane, and may coalesce if numerous.

In females the condition appears to be self-limiting, and there is no evidence that it causes infertility. It is in the male that the condition is more commonly seen, because it can progress to balanitis with marked swelling of the distal prepuce and secondary bacterial infection. If detected early it is possible that hydrotherapy and the administration of antibiotic may help in clearing up the condition. However, it is often the case that the condition is seen when the animals are in rut, when they are extremely aggressive, making frequent hydrotherapy dangerous for both handler and patient.

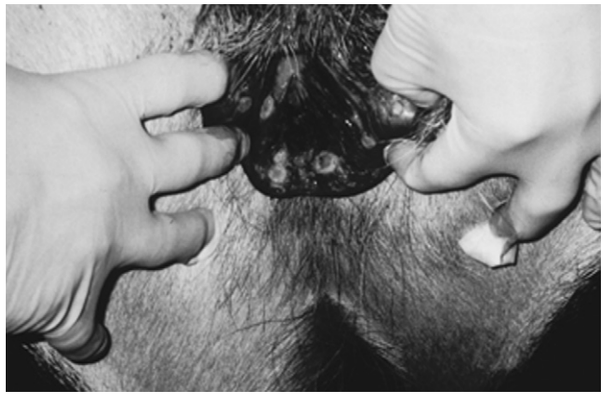

Fig. 141-2 Ulcerative lesions in the vulva of a female wapiti.

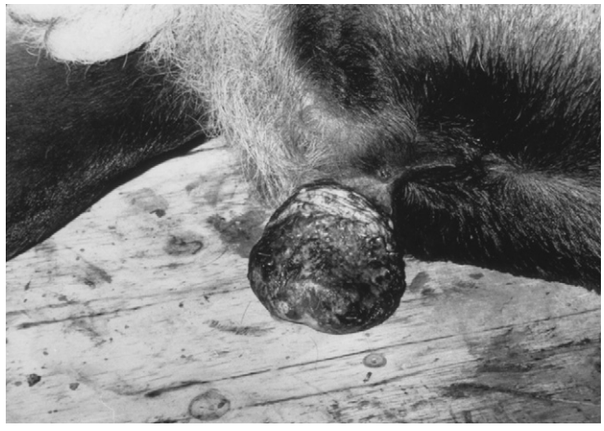

Fig. 141-3 Posthitis in a wapiti stag. The preputial tip is swollen and has become infected and necrotic. (Photograph by Colin Mackintosh; from Haigh JC, Hudson RJ: *Farming wapiti and red deer.* St. Louis: Mosby, 1993.)

Many cases in males are seen when there is some degree of preputial prolapse, often accompanied by swelling and bacterial infection. Because of the dependent position of the distal prepuce these cases are seldom resolved with simple measures, and require amputation of the affected portion. This can be accomplished surgically or with the use of compression. For the latter the animal is restrained in a chute. The mucus membrane is exteriorized until healthy tissue is found on the inner surface, and then the compression ring is applied. At least three materials have been used. Tight bandage or a rubber castrating ring has been placed over the tissue while a large syringe case (its end cut off) was held in the lumen of the sheath, or equipment designed to deal with rectal prolapse (Callicrate Smart Bander, No-bull Enterprises, St. Francis, Kansas) was used. After a few days the affected part, which has undergone ischemic necrosis, falls away and recovery is usually complete.

A disease of European bison (*Bison bonasus*) that is characterized by a chronic necrotizing inflammation of

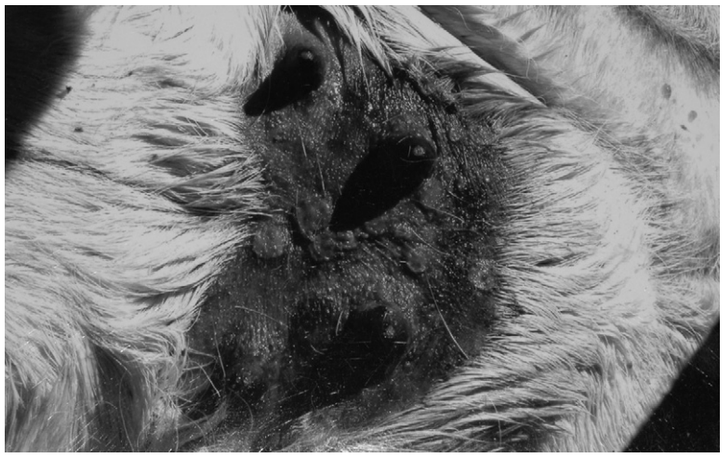

Fig. 141-4 Acute poxlike lesions on the udder of a reindeer. (Courtesy Jerry C. Haigh.)

the prepuce and penis has been observed since 1980.[15] More than 30% of male bison in the population were affected in 1997. The histologic and microbiologic results indicate a necrobacillosis with *Fusobacterium necrophorum* as the etiologic agent. The authors suggest that there are probably a variety of factors contributing to the etiology and pathogenesis, and presumably several agents are involved and interact synergistically. This condition has not been reported in North American bison.[15]

Five cases of phimosis, all in related axis deer (*Axis axis*) in a zoo, have been observed. One was treated surgically.[16]

Necrosis of the distal part of the penis has been seen in two red deer. Clinical signs in one of these included severe abdominal pain, and ataxia and paresis of the hind limbs. Pathologic examination showed necrosis of the distal part of the penis, with marked swelling.[16]

MISCELLANEOUS

The author has seen two cases of mummified fetus in wapiti and one in a moose. The moose mummy was delivered within minutes of the birth of a normal, live calf. Both wapiti cases were resolved by the use of two doses of prostaglandin $F_{2\alpha}$ given 30 days apart. In one closely observed instance a vaginal discharge of mucopurulent material occurred over several days after the second injection. The animal did not conceive the following autumn, but went on to calve in subsequent years.[3]

Urinary calculi leading to urethral occlusion has been seen in wapiti at the Western College of Veterinary Medicine. Treatment by urethrotomy alleviated the condition, but is not likely to be of any long-term use. In cattle, treated cases soon proceed to slaughter. In wapiti any thought of keeping the animal for the production of velvet antler in future years has to be tempered by the fact that long-term survival is not likely.

Neoplastic conditions are occasionally seen.

An outbreak of an acute disease of the udder was seen in Mongolian reindeer in the late summer of 2004. Seventeen cases that involved the development of numerous pox-like lesions on the hairless ventral portion were seen (Fig. 141-4). These animals were being milked at the time, and some of the herders developed similar lesions on their hands. A single biopsy was taken from the edge of a fresh lesion, but unfortunately, no diagnosis was possible. Most of the animals were treated conservatively with the application of a zinc-oxide paste. The lesions regressed over the course of about 2 weeks, and no further cases have been reported by the herders.[17]

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